OVERVIEW OF CARCINOGENIC MECHANISMS FOR 109 AGENTS KNOWN TO CAUSE CANCER IN HUMANS

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INTRODUCTION

In 1970, the International Agency for Research on Cancer (subsequently referred to as IARC) began a process of evaluating the carcinogenic risk of chemicals to humans. This process identified candidate agents and conducted a comprehensive review of the available laboratory, animal and human evidence of the carcinogenetic potential. The process of review was extensive, involving expert committees (Working Groups) and an exhaustive examination of the available literature of the human and animal evidence of increased cancer risk related to each agent. As part of its evaluation of potential carcinogenic agents, the working groups reviewed the available data on mechanistic pathways by which such agents may exert a carcinogenic effect. The process developed by IARC to prepare these reviews is documented in each of the published monographs; for details of the process as currently implemented by IARC, see the preamble section of Volume 100A of the Monograph series [1, pp. 9-31].

The reports from these IARC Working Groups have been published by IARC in a series of monographs officially called: the IARC Monographs on the Evaluation of Carcinogenic Risk in Humans. The first monograph was published in 1972 and examined 19 organic and inorganic substances. Since that initial volume, a total of 106 monographs have been published (up to 2014), addressing a wide range of potentially carcinogenic agents including pharmaceuticals, chemicals, radiation, personal habits and biological agents. These monographs have identified 109 agents that have been classified as Group 1 human carcinogens. These 109 agents will be the focus of the current chapter.

In 2009, IARC began preparation of a special monograph: Volume 100. It was published in six volumes in 2012. These volumes examined agents in six broad groups:

- Pharmaceuticals (Volume 100A [1]),
- Biological agents (Volume 100B [2]),
- Arsenic, Metals, Fibres, and Dusts (Volume 100C [3]),
- Radiation (Volume 100D [4]),
- Personal Habits and Indoor Combustions (Volume 100E [5]) and
- Chemical Agents and Related Occupations (Volume 100F [6]).

The Volume 100 monographs examined the available scientific evidence for the 107 agents identified in Volumes 1-99 of the IARC Monographs as known human carcinogens by the Agency. These are the complete list of Group 1 Human Carcinogens: agents for which there is sufficient evidence of the potential to increase human cancer risk, according to the criteria used by the IARC. Between the publication of Volume 100 and 2014, six additional monographs have been published which have identified a further two Group 1 agents, one each in Volume 105 [7] and Volume 106 [8].

Subsequent to the publication of the Volume 100 monographs, an international project was undertaken to perform a detailed analysis of the data on mode of action and tumor site concordance for the Group 1 agents included in those volumes 100, plus the two additional Group 1 agents identified in volumes 101 through 106. Each of the IARC volumes contains an assessment of the mode of action of the 109 Group 1 human carcinogens. The overall aim of the international project was to develop an enhanced understanding of the mechanism and dose–

response relationships for carcinogenic agents of different types and thus to provide a better basis for assessing the human cancer risks associated with these agents. The project also included an evaluation of tumor site concordance between animals and humans to assist in determining the relevance of animal data for human risk assessment.

The purpose of the current chapter is to provide a narrative synopsis of the mechanistic data for each of the 109 agents identified as Group 1 human carcinogens through the IARC monograph review process, up to and including volume 106. For the 107 Group 1 agents identified in IARC Monographs Volume 100A through 100F, we used the mechanistic information contained within the relevant Volume 100 publication. For agents identified in Volumes 101 through 106, we used the mechanistic information contained in those volumes.

This chapter is based on expert opinion concerning the available primary data. We have not attempted to conduct a full systematic review or scientific critique of the evidence. The IARC Working Groups have already undertaken this process; the synopses presented here build on that foundation. The extent of the literature supporting the mechanistic data for these 109 agents precludes the inclusion of full citations to support the narrative overviews. Supporting references, as well as a more exhaustive discussion of the mechanistic summaries can be found in the primary IARC Monographs associated with each agent. A reference to the appropriate IARC Monograph volume is provided in the agent-specific sections.

The understanding of carcinogenesis, and the mechanisms of action of specific agents, has developed since the Volume 1 monograph was published in 1972. In preparation of Volume 100, the Working Groups updated the modes of action as described in the earlier volumes that discussed each agent. This chapter will use the Volume 100 mechanistic summaries as the foundation. The Volume 100 material should be current with literature published up to about 2010. However, in recognition that further mechanistic work, particularly relating to the role of epigenetics and miRNAs, will have occurred since the final meeting of the IARC Volume 100 Working Groups, we extended the literature to address mechanistic publications occurring after that date.

This chapter was prepared to provide background to a more comprehensive evaluation of the mechanisms of human cancer conducted by the IARC Working Group on Tumour Concordance and Mechanisms of Carcinogenesis, which was convened to guide the preparation of the IARC Scientific Publication in which this article appears.

The IARC Working Groups which produced Volumes 100A-100F, created a list of mechanistic terms from the agents that they reviewed. Robert Baan used this to create tables that summarized the information related to the final IARC Classification, tumor site and carcinogenic mechanisms for each agent reviewed by the Working Groups. Members of the IARC Volume 100 Working Group met in May, 2012 and used this information to create a list of 24 categories of carcinogenesis. Later that year (November, 2012), a second meeting of the Working Group generated the list of 10 mechanistic characteristics. The 24 and 10 mechanistic groupings are described in more detail in companion chapters in the monograph.

Number of Group 1 human carcinogens reported in chapters

The chapters discussing carcinogenic mechanisms and animal-human concordance are based on a consideration of the agents classified as Group 1 Human carcinogens through the IARC

Monograph programme. A full list of all agents reviewed through the IARC monograph programme, and their Group classification, can be found at the following URL: http://monographs.iarc.fr/ENG/Classification/ClassificationsGroupOrder.pdf. Although working from this common list, the chapters report on slightly different numbers of agents. This section explains the differences.

The material on carcinogenic mechanisms was based on Group 1 agents identified up to and including Volume 106. There are 109 agents identified from these volumes. However, within the Volume 100 monographs, these agents were commonly grouped into chapters that discussed similar agents (e.g. isotopes of radon, radium and related radionucleotides were combined into one chapter). There are 84 chapters. Since agents within a chapter share carcinogenic mechanisms, some analyses are based on this smaller number of agents.

The animal-human concordance analyses extended the selection of agents to include Volumes 107 through 109 (4 additional agents), which gives a total of 113 Group 1 agents. However, volume 107 classified 'PCB's' as a broad category as a Group 1 agent. Volume 100F had already identified one PCB (3,4,5,3',4'-Pentachlorobiphenyl or PCB-126) as a group 1 agent. The concordance analysis re-grouped this agent into the inclusive PCB category of Volume 107, thus reducing the number of Group agents to 112. For analytical work, some agents were excluded; this is detailed in the relevant chapter.

METHODS

All agents classified by IARC as Group 1 human carcinogens in Volumes 100A through 100F and Volumes 101 through 106 of the IARC Monographs on the Evaluation of Carcinogenic Risk in Humans were identified. For each agent from Volume 100, the mechanistic material presented in the relevant chapter of the appropriate Volume 100 chapter was reviewed by three authors (NJB, MA-Z and MB) to identify core carcinogenic mechanisms. The information reviewed was mainly contained in Section 4 of the monograph chapters. A similar process was used for agents identified from Volumes 101 thorough 106 based on the mechanistic review chapters in those reports. For each agent, a textual summary was written describing the mechanism of carcinogenesis.

A literature search was conducted to identify key mechanistic information that post-dated the IARC Working Group reviews. For each agent, a PUBMED search was conducted to identify publications that provided evidence of a potential carcinogenic mechanisms and which had not been included in the IARC review. For any endpoint or mechanism that was found to be linked to the carcinogen and which was had not already been identified in the narrative synopsis, additional information was added to the summary. Up to three articles supporting the new mechanisms were identified and referenced in the summary. The results of this review were included in the final section of each synopsis report. Where no additional information was identified, this supplemental section is not presented.

A narrative summary of the carcinogenic mechanistic information is presented for each of the 109 Group 1 human carcinogens. Each agent is assigned a separate section of this chapter. The section heading identifies the Group 1 carcinogen using the nomenclature of the Group 1 carcinogen listed in the corresponding IARC monograph. The name is followed by a list of IARC monographs that have discussed the carcinogen. The first paragraph in most sections is a short quotation from the associated IARC monograph, providing the monograph's overview of the

broad carcinogenic mechanisms involved for the agent under review. This is followed by a summary of the mechanistic information. No primary references are provided since this material is a synopsis of the well-referenced IARC monographs. In several cases, a single IARC chapter discussed multiple agents that were classified separately as distinct Group 1 carcinogens. Each of these agents is listed in a separate section in this chapter. However, the mechanistic summary is not repeated but rather references the discussion presented for a representative agent from the chapter. Each section concludes with a short presentation of the additional mechanistic information (if any) that was identified through a review of any relevant literature that post-dated the publication of the IARC Volume 100 monographs.

The summary mechanistic tables produced by the IARC 100 Working Groups were adapted for inclusion in this chapter (Tables 1 – 5); since the Working Group responsible for Volume 100D (radiation) only reported tumor site and carcinogen classification but did not identify carcinogenic mechanisms, no table was created for that volume. Only information related to Group 1 Human Carcinogens is included. We excluded information in the Working Group tables that was not directly related to carcinogenic mechanisms.

RESULTS

The IARC monographs up to volume 106 identify 109 unique agents as Group 1 human carcinogens. There is some inconsistency in the approach to determining how agents were listed by IARC. For example, various isotopes of Radon are each classified as separate Group 1 carcinogens but, two distinct chemicals associated with smoking (NNN and NNK) are combined as one Group 1 carcinogen entry rather than separated out as distinct entries. PCBs are listed as a global category rather than separated out as distinct congeners. In addition, some carcinogens reflect composite exposures (e.g. painters) where subjects are exposed to multiple potential carcinogens while others are narrowly focused on a specific chemical (e.g. benzene).

The Results material presents the qualitative narrative summaries from the IARC monographs for each of the Group 1 carcinogenic agents identified by IARC. The agents will be presented in the order in which they appeared in Volume 100, starting with Therapeutic agents (Volume 100A) and ending with Chemical Agents and Related Occupations (Volume 100F). Agents identified from Volumes 101 through 106 are reported after the information for Volume 100F.

VOLUME 100A: THERAPEUTIC AGENTS

Busulfan (Volume 4 & Supplement 7)

'Busulfan is a direct-acting alkylating agent that is carcinogenic via a genotoxic mechanism.' (Busulfan [1, p. 43]).

Busulfan (1,4-Butanediol dimethanesulfonate) is a direct-acting bifunctional alkylating agent. It was widely used for the treatment of chronic myeloid leukemia prior to release of Imatinib. Busulfan causes acute myeloid leukemia.

The primary mechanism of carcinogenesis is through genotoxicity. Busulfan binds covalently to cellular macromolecules including DNA, RNA, and proteins. Consequently, it is capable of producing mono-adducts, intrastrand cross-links, and DNA–protein cross-links. In-vitro testing with human and rodent cells treated with Busulfan induced: chromosomal aberrations, sister chromatid exchange, and mutations. Patients treated with busulfan for chronic myeloid

leukaemia were found to have increased frequencies of sister chromatid exchange and chromosomal aberrations in their peripheral blood lymphocytes. In-vivo treatment of rodents with busulfan induced dominant lethal mutations, and increased the frequency of chromosomal aberrations or micronuclei in bone marrow, intestine, embryonic liver, and germ cells. Evidence suggests that busulfan directly induces losses or deletions affecting chromosomes 5 or 7 (e.g. loss of heterogeneity for p53). Chromosomal alterations and deletions have been reported in a variety of experimental models and in human lymphocytes.

Chlorambucil (Volumes 9, 26 & Supplement 7)

'Chlorambucil is a direct-acting alkylating agent that is carcinogenic via a genotoxic mechanism.' (Chlorambucil [1, p. 53]).

Chlorambucil is an antineoplastic agent used primarily to treat several leukemias and lymphomas. Chlorambucil has been causally linked to acute myeloid leukemia.

Chlorambucil forms covalent DNA adducts. Chlorambucil contains two chloroethyl groups, one of which can react with the N7 position of guanine or adenine. The second chloroethyl group can then react with cellular proteins or with a DNA base to form a stable inter-strand DNA cross-link, leading to mitotic delay. Failure to repair the adducts can lead to mutations.

Chlorambucil has been tested for genotoxicity in several short-term assays *in vitro* and *in vivo*. It has been shown to be mutagenic in bacteria after metabolic activation. It causes a range of genetic damage including: gene conversion in yeast, sex-linked recessive mutations in Drosophila, mutations in Chinese hamster ovary cells, and clastogenic effects in human lymphocytes in vitro, and in animals *in vivo*. Exposure to chlorambucil increases the frequency of micronucleus induction and chromosomal aberrations in rat bone marrow and spleen *in vivo.*

Methyl-CCNU (Volumes 26, 42 & Supplement 7)

'Methyl-CCNU is a direct-acting alkylating agent that is carcinogenic via a genotoxic mechanism.' (Methyl-CCNU [1, p. 60]).

Methyl-CCNU (1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea) is an anti-neoplastic agent that was used as an investigational drug to treat various cancers. It causes acute myeloid leukemia.

Methyl-CCNU is a bifunctional antineoplastic agent that is carcinogenic through a genotoxic mechanism. Genotoxicity arises from electrophilic alkylating metabolites produced by spontaneous chemical decomposition and CYP-P450 mediated metabolism. These induce alkylation and carbamoylation[1] of cellular macromolecules, including DNA and protein. Alkylation can produce G-C crosslinking in DNA. Carbamoylation of cellular proteins is believed to inhibit DNA-repair processes.

Genotoxicity has been demonstrated in a range of short-term assays. This includes the induction of DNA adducts in the bone marrow, spleen and colon of treated mice, and in the kidney, liver and lung of treated rats. Micronuclei are more frequently seen in the bone-marrow erythrocytes of mice treated. Chromosomal aberrations, micronuclei, sister chromatid exchange, and DNA strand breaks have been reported in human or rodent cells treated in vitro with Methyl-CCNU.

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[1] Carbamylation is a posttranslational modification of proteins resulting from the non enzymatic reaction between isocyanic acid and specific free functional groups

Patients treated with methyl-CCNU show increased frequency of sister chromatid exchange and elevated levels of chromosome aberrations in peripheral blood lymphocytes.

Cyclophosphamide (volumes 9 & 26, Supplement 7)

'Cyclophosphamide, after its bioactivation to alkylating metabolites, is carcinogenic via a genotoxic mechanism'. (Cyclophosphamide [1, p. 82])

Cyclophosphamide is an antineoplastic agent that is widely used in cancer treatment for its immunosuppressive properties and has been heavily researched concerning metabolism and carcinogenicity. The parent compound does not display carcinogenicity. Rather, it requires metabolic activation in the liver. The two primary metabolites which have carcinogenic potential are: phosphoramide mustard and acrolein. Cyclophosphamide causes cancer of the bladder and acute myeloid leukemia.

Phosphoramide mustard binds covalently to DNA, producing various types of DNA adducts. Comet assay testing has revealed evidence of single-strand DNA breakage and related lesions. Several biomarkers of genotoxicity have been detected more frequently in patients treated with cyclophosphamide. *In vitro* testing has revealed a wide range of mutagenic effects in both human cells (chromosomal aberrations, sister chromatid exchange, and DNA damage) and rodent cells (morphological transformation, chromosomal aberrations, sister chromatid exchange, mutation, and unscheduled DNA synthesis (UDS)). These effects were enhanced following incubation in *S9* liver fractions. *In vivo* testing in mice found evidence of DNA adduct formation and, in rodents, dominant lethality, chromosomal aberrations, micronuclei, sister chromatid exchange, mutations, and DNA damage.

Acrolein, in addition to displaying DNA binding, has been linked to the production of cystitis, which contributes to carcinogenesis through a promotion effect.

Etoposide in Combination with Cisplatin and Bleomycin (Volumes 26 & 76)

'Etoposide in combination with cisplatin and bleomycin is carcinogenic via a genotoxic mechanism.' (Etoposide in combination with cisplatin and bleomycin [1, p. 101])

Etoposide, cisplatin and bleomycin are frequently administered to patients as a form of combined chemotherapy. The three agents have different mechanisms of action and carcinogenic potential. Etoposide has been the most extensively studied and has the clearest carcinogenic profile. Etoposide in combination with cisplatin and bleomycin causes acute myeloid leukaemia.

All three of these drugs interfere with the ability of DNA polymerase to synthesize a cDNA strand. Etoposide binds to topoisomerase II interfering with DNA replication. Topoisomerase II reduces DNA tangles and supercoils by producing double-strand DNA breaks that are then re-ligated. The etoposide-topoisomerase IIα complex interferes with DNA re-ligation, enhancing the production of DNA double-strand breaks. These complexes can also directly block the advancing DNA replication fork. Cisplatin and bleomycin also enhance double-strand breaks. Each of these individual drugs induce sister chromatic exchange and aneuploidy.

Etoposide has also been shown to induce chromosomal breakages, rearrangements, and translocations within the MLL gene in experimental systems (e.g., mouse embryonic stem cells and in haematopoietic CD34+ cells in culture, including human long-term repopulating haematopoietic stem cells).

Etoposide (Volumes 26 & 76)

'Etoposide in combination with cisplatin and bleomycin is carcinogenic via a genotoxic mechanism.' (Etoposide in combination with cisplatin and bleomycin, IARC Volume 100A, p101)

Etoposide in combination with cisplatin and bleomycin causes acute myeloid leukaemia; the combination is classed as a group 1 human carcinogen. However, etoposide as a separate agent was also classified by IARC as a group I carcinogen. Mechanistic information provided the primary basis for this classification. A summary of the carcinogenic mechanism for etoposide is presented in the previous section but is repeated here.

Etoposide binds to topoisomerase II interfering with DNA replication. Topoisomerase II reduces DNA tangles and supercoils by producing double-strand DNA breaks that are then re-ligated. The etoposide-topoisomerase IIα complex interferes with DNA re-ligation, enhancing the production of DNA double-strand breaks. These complexes can also directly block the advancing DNA replication fork. It induces sister chromatic exchange and aneuploidy.

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Melphalan (Volume 9 and Supplement 7)

'Melphalan is a direct-acting alkylating agent that is carcinogenic via a genotoxic mechanism' (Melphalan, IARC Volume 100A, p 113)

Melphalan is used in the treatment of several neoplasms, including multiple myeloma. Melphalan causes acute myeloid leukemia.

Melphalan (4-[bis(chloroethyl)amino]phenylalanine) is a direct-acting, bifunctional, alkylating agent that binds to cellular macromolecules including DNA, RNA and proteins. It has been shown to produce various types of DNA adducts and to induce DNA strand cross-linking. Adducts and cross-linking have been observed in *in vitro* rodent studies and in patients treated with melphalan. The later have been noted particularly in the genes *TP53* and *N-RAS*. *In vitro* and *in vivo* studies have found an increased frequency of dominant lethal mutations, chromosomal aberrations, micronuclei, and DNA strand breaks in rodents. Similar effects have been seen in human cells treated *in vitro.* Mutations of the HPRT gene have also been noted. Human patients treated with melphalan have also shown chromosomal aberrations and sister chromatid exchange in peripheral lymphocytes.

MOPP (Supplement 7; two of the component agents were reviewed in Volumes 9 & 26)

'The MOPP combination as well as individual components, except for prednisone, are genotoxic, and induce cancer via a genotoxic mechanism.' (MOOP, IARC Volume 100A, p 126)

MOPP refers to a four drug chemotherapeutic regimen composed of: mechlorethamine, oncovin, procarbazine, and prednisone. The individual agents have been subject to separate IARC reviews. MOPP use has been superseded by more recent therapeutic alternatives. MOPP causes cancer of the lung and acute myeloid leukemia.

Mechlorethamine (Chlormethine, Nitrogen Mustard) is a bifunctional alkylating agent that binds to DNA and produces mono-adducts and cross-linkages. *In vitro* studies show that it induces

chromosomal aberrations, sister chromatid exchange, and unscheduled DNA synthesis in both rodent and human cells. *In vivo* studies in mice produced dominant lethal mutations and micronuclei in the bone-marrow cells. Chromosomal aberrations were noted in one study of treated human patients.

Oncovin (vincristine sulphate) is a Vinca alkaloid that interferes with microtubule assembly and spindle formation, blocking cell replication. It induced micronuclei in the bone-marrow cells of mice and hamsters treated *in vivo*, and aneuploidy and transformation in hamster embryo cells but did not induce sister chromatid exchanges or structural chromosomal aberrations.

Procarbazine is a methylhydrazine derivative whose carcinogenicity requires metabolism to a reactive intermediate (a methyl diazonium cation). This active agent methylates DNA. *In vivo* studies have found that it induces micronuclei and structural chromosomal aberrations in mice, sister chromatid exchange in mice and Chinese hamsters, and causes DNA damage in rodents.

Prednisone is a synthetic glucocorticoid with multiple modes of action. It produces a range of anti-inflammatory and immunosuppressive effects. There is no evidence of mutagenicity or direct DNA damage.

Several researchers have examined the carcinogenicity of the MOPP combination therapy. Their results confirm the findings described above for the individual components.

Tamoxifen (Volume 66)

'There is strong evidence that in rat liver, tamoxifen is a genotoxic carcinogen through a pathway involving α-hydroxylation [to produce 4-hydroxytamoxifen], sulfation of th[is] α-hydroxy metabolite, and subsequent DNA-adduct formation. Evidence for the role of this pathway in induction of human endometrial tumours is less compelling; rather, the data suggest that the carcinogenicity of tamoxifen is associated with an oestrogen-receptor-dependent pathway.' (Tamoxifen, IARC Volume 100A, p155)

Tamoxifen is a first-line drug for the treatment of metastatic female breast cancer in post-menopausal women. In addition to chemotherapy uses, it has been suggested as a chemopreventive agent for women at high risk of breast cancer. Tamoxifen causes endometrial cancer.

IARC Volume 100A concluded: *'The available evidence indicated that tamoxifen is both a genotoxic carcinogen and a tumour promoter in rat liver, and that humans are likely to be less susceptible to the genotoxicity of the drug (p148).*

Evidence for a genotoxic carcinogenesis in humans is not compelling. There are conflicting reports on the formation of tamoxifen-DNA adducts in humans, with some groups finding adducts in endometrial, colon and white blood cells. Animal studies have been more consistent in finding adducts in rat and mouse liver. There is a suggestion that the production of adducts is linked to a minor phase I metabolic pathway (sulfotransferase-mediated sulfation, specifically by the STA2 isoform of SULT2A1) that may explain the discrepant results. Most studies have not detected DNA adducts in the uterus and other extra-hepatic tissues from rats.

Tamoxifen induces micronuclei in metabolically proficient human cells and causes aneuploidy and chromosomal aberrations. Moreover, both tamoxifen and 4-hydroxytamoxifen cause mutations in the *lacI* reporter gene and the cII gene in the livers of Big Blue® transgenic rats.

A non-genotoxic pathway is supported for human carcinogenesis. Tamoxifen is a selective oestrogen receptor modulator. In endometrial endothelium, Tamoxifen acts as an agonist, stimulating cellular proliferation. Evidence suggests that the genes targeted by tamoxifen activation of the oestrogen receptor differ from those stimulated by oestrogen. This mechanism may be responsible for the differential action of tamoxifen in distinct tissues and may contribute towards carcinogenicity.

Updated Information

Tamoxifen has been found to induce changes in DNA methylation patterns in vivo in humans and animals [9,10,11], and to induce changes in the expression patterns of mRNAs in the liver of treated rats [12]. Tamoxifen also has been found to induce changes in cell proliferation as well as expression of individual telomerase activity in human carcinoma cell lines [13].

Thiotepa (Volumes 9 & 50; Supplement 7)

'Thiotepa is an alkylating agent that is carcinogenic via a genotoxic mechanism. ' (Thiotepa, IARC Volume 100A, p 168)

Thiotepa (N,N'N'-triethylenethiophosphoramide) was used for chemotherapy but has been largely replaced by newer agents. Thiotepa causes leukemia.

Thiotepa is rapidly metabolized to triethylenephosphoramide (TEPA). Thiotepa and TEPA are alkylating agents and appear to contribute to the carcinogenic profile of thiotepa. There is strong evidence that both thiotepa and TEPA form DNA adducts and DNA cross-linkage. Thiotepa is cytotoxic, producing mutations. The rate of adduct production is elevated if DNA-repair mechanisms are blocked. *P53* loss also exacerbates the mutation effect. Thiotepa has been found to induce micronuclei and chromosomal aberrations in bone marrow of mice and rats, and also causes chromosomal aberrations and sister chromatid exchanges in Rhesus monkeys. Patients receiving therapy with thiotepa displayed higher levels of chromosomal aberrations in peripheral lymphocytes than untreated patients.

Treosulfan (Volume 26; Supplement 7)

'Treosulfan is carcinogenic via a genotoxic mechanism.' (Treosulfan, IARC Volume 100A, p 173)

Treosulfan ((2S,3S)-2,3-Dihydroxybutane-1,4-diyl dimethanesulfonate) is used in the treatment of ovarian cancer. Treosulfan causes acute myeloid leukemia.

Treosulfan is a pro-drug that is converted non-enzymatically to a mono-epoxide and a diepoxide. Evidence suggests that the carcinogenic properties of treosulfan are derived from these metabolites. Treosulfan is a bifunctional alkylating agent that alkylates DNA and creates interstrand crosslinks. It is mutagenic in *S. typhimurium* strains TA100 and TA1535 in the absence of metabolic activation and in Chinese hamster cells. Few *in vivo* studies have been reported although two studies reported the treosulfan induced micronuclei in mouse bone marrow.

Diethylstilbestrol (Volumes 6, 21 & 42; Supplement 7)

'It is likely that two or more of these factors [see later] in combination are responsible for the carcinogenic effects of diethylstilbestrol; oestrogen-receptor mediated effects and genotoxicity conceivably both being involved, while other factors may be contributory. The early developmental changes in the female and male genital tract caused by exposure to diethylstilbestrol in utero or –

in rodents – neonatally, may result in epigenetic events that create a tissue and cellular environment conducive for the mechanisms responsible for the transplacental carcinogenic effects of diethylstilbestrol in humans and animals.' (Diethylstilbestrol, IARC Volume 100A, p 206)

Diethylstilbestrol was used to prevent miscarriage. It has also been used to treat prostate cancer and as a livestock growth stimulant. It is no longer commercially available in the USA. Diethylstilbestrol causes cancer of the breast. It also causes clear cell adenocarcinoma of the cervix and vagina in women who were exposed to the drug *in utero* as a fetus.

Understanding the mechanism of carcinogenesis for diethylstilbesterol is challenging. While this drug can cause cancer in women exposed to it as adults, one unusual feature is that *in utero* or pre-natal exposure can cause cancer in the offspring of the exposed woman. There is some evidence that such exposure can affect the grandchildren of the exposed woman. This complicates consideration of carcinogenic mechanisms.

IARC Volume 100A provides a very lengthy discussion of diethylstilbestrol. Their synthesis of mechanisms of action suggests that multiple pathways are likely involved. Five categories of mechanisms were explored.

Direct Genotoxicity

In vivo studies have shown some evidence for DNA adduct formation. However, it appears that these adducts may be oxidative-stress-generated lipid–hydroperoxide–and malondialdehyde–DNA adducts. Aneuploidy, sister chromatid exchange, chromosomal aberrations and micronuclei have been reported in some species and tissues but the evidence is conflicting. *In vitro* studies have found evidence for the production of aneuploidy. Diethylstilbestrol inhibited the polymerization of microtubules in human fibroblasts and prostate cancer cells. This may underlie the production of aneuploidy. There is little evidence that diethylstilbestrol produces mutations. There is evidence that mitochondria might be a target for diethylstilbestrol. DNA mitochondrial adducts have been detected and there is evidence of oxidative metabolism of diethylstilbestrol by mitochondria. Mitochondria also are known to express functional ERα and ERβ receptors.

Cell Proliferation and Apoptosis

Diethylstilbestrol treatment increases the mitotic rates and stimulates cellular proliferation for selected tissues. Diethylstilbestrol can immortalize primary animal embryo cells in vitro and transform human breast cell lines.

Immune Modulatory Effects

Several studies have found evidence for immune system modulation. This is dose-dependent, appears mediated by the thymus and differs in males and females.

Oestrogen-Receptor Mediated Effects

In utero exposure led to long term alterations in the hormonal response of reproductive tissues in both male and female offspring. These effects were modified in animal models where oestrogen receptor levels could be adjusted.

Effects on gene expression (hormonal imprinting)

In utero exposure produced persistently elevated expression of several genes, including proto-oncogenes such as *c-fos* and *c-myc*. There was evidence of hypomethylation of the promoter regions of these genes.

Updated Information

Diethylstilboestrol can affect the epigenetic mechanisms by changing DNA methylation patterns [14,15] inducing histone deacetylation [16] and the down-regulation of expression of micro RNAs [17].

Oestrogen-only Menopausal Therapy (Estradiol: Volume 6 & 21; Supplement 7. Post-menopausal oestrogen therapy: Volume 72; Supplement 7)

'Receptor-mediated responses to hormones are a plausible and probably necessary mechanism for oestrogen carcinogenesis. In addition, there is support for a genotoxic effect of estrogenic hormones or their associated by-products such as reactive oxygen species. .... Current knowledge does not allow a conclusion as to whether either of these mechanisms is the major determinant of oestrogen induced cancer. It is entirely possible that both mechanisms contribute to and are necessary for oestrogen carcinogenesis.' (Oestrogen-only menopausal therapy, IARC Volume 100A, p 241)

Conjugated oestrogens refer to a group of at least eight related compounds. Estradiol is the most potent of these agents. They are most commonly used for hormonal replacement in women who have undergone menopause mainly as a result of having a hysterectomy. Epidemiological evidence has established oestrogen as a cause of endometrial and ovarian cancer and likely of breast cancer.

There is little evidence that oestrogens directly affect DNA. However, the major metabolic pathway for oestrogens is via CYP1A1/1B1 that produces *o*-quinone compounds that can produce DNA adducts. Several adducts can be produced, with varying impact on DNA replication. The further metabolism of oestrogens leads to redox cycling and the generation of reactive oxygen species that are known to damage DNA. This mechanism is hypothesized to underpin oestrogen induced tumour initiation/promotion.

There is some evidence for estradiol inducing DNA strand breaks, sister chromatid exchange, and chromosomal aberrations in animal and human cells. Aneuploidy has also been seen in animals. However, mutations are not consistently seen during *in vitro* studies.

Oestrogen therapy increases cellular proliferation through stimulation of nuclear oestrogen receptor mediated signalling pathways. Oestrogen receptors (ERα and ERβ) have recently been identified in the mitochondria and may be involved in deregulation of mitochondrial bioenergetics and creation of oxidative stress.

Updated Information

Oestrogen-only replacement therapy has been shown to increase DNA methylation [18,19], and to increase telomerase activity [20,21].

Combined Oestrogen-progestogen Menopausal Therapy (Volumes 72 & 91; Supplement 7)

'Current knowledge indicates that hormone receptor-mediated responses are a plausible and probably necessary mechanism for hormonal carcinogenesis by combined oestrogen-progestogen

menopausal therapy . There is also support for the potential involvement of genotoxic effects of combined oestrogen–progestogen menopausal therapy estrogenic hormones or their associated metabolic by-products including the formation of DNA adducts, and reactive oxygen species that damage DNA.' (Combined oestrogen-progestogen menopausal therapy, IARC Volume 100A, p 277)

Combined oestrogen-progestogen therapy replaced oestrogen-only therapy in an attempt to avoid the increased risk of endometrial cancer associated with oestrogens. Unfortunately, the epidemiological evidence indicates that the combined therapy also causes both breast and endometrial cancer.

The potential mechanisms of action for oestrogen were discussed in the previous section.

Progestogens, including those used for combined oestrogen–progestogen menopausal therapy, appear to have the capacity to stimulate cell proliferation in the breast while they inhibit proliferation in the uterus. The magnitude of these effects varies for different synthetic progestogens, with a suggestion that medroxyprogesterone acetate is very active.

There is no evidence linking progestogen to direct DNA damage. An *in vitro* cell culture study using human breast cancer cells in a progesterone treated growth medium showed that the cells released paracrine factors which stimulated VEGF receptors and induce proliferation of endothelial cells and breast cancer cells.

There is a suggestion that progestogen, when taken in combination with oestrogens, reduces the carcinogenic potential of oestrogen. The mechanism for this effect is unclear. There is suggestive evidence that binding of progesterone to androgen receptors might be part of the mechanism. This is based on the hypothesis that the signalling pathways are linked, leading to some suppression oestrogen-induced functions.

Updated Information

Oestrogen-only replacement therapy has been shown to increase DNA methylation [18,19], and to increase telomerase activity [20,21].

Combined Oestrogen-progestogen Oral Contraceptives (Volumes 72 & 91; Supplement 7)

'Hormone-receptor-mediated responses are probably a necessary mechanism for hormonal carcinogenesis by combined oestrogen–progestogen oral contraceptives. .... Because oestrogen mediates the expression of progesterone receptors, the presence of oestrogen in these combined oestrogen–progestogen oral contraceptives may be essential for progestogen mediated cell proliferation. .... There is also support for the involvement of genotoxic effects of the metabolic by-products of estrogenic hormones in combined oestrogen–progestogen oral contraceptives or of the reactive oxygen species generated in response to them.' (Combined oestrogen-progestogen contraceptives, IARC Volume 100A, p 310)

Combined oestrogen–progestogen oral contraceptives cause cancer of the breast, in-situ and invasive cancer of the uterine cervix, and cancer of the liver.

The use of an oestrogen-progestogen combination as an oral contraceptive differs from the use of these same drugs for post-menopausal treatment mainly through differences in dosage, patterns of administration and the age of the patients. The active chemical agents are the same. Therefore, potential carcinogenic mechanisms are also the same.

Since these agents have been discussed in the previous two sections, the information will not be replicated here.

Azathioprine (Volume 26; Supplement 7)

'Azathioprine is carcinogenic via two mechanisms:

- *As an immunosuppressant, it is associated with post-transplant lymphoproliferative disorders that generally have a viral aetiology;*
- *Because it causes 6-thioguanine to accumulate in patients' DNA, it also contributes to cancer development by DNA damage'. (Azathioprine, IARC Volume 100A, p328-9)*

Azathioprine (6-[(1-Methyl-4-nitro-1H-imidazol-5-yl)sulfanyl]-7H-purine) is used as an adjunct immunosuppressant for patients undergoing renal transplantation. It is a pro-drug which is converted to 6-mercaptopurine which is itself used in treating acute lymphocytic leukemia. It causes squamous cell carcinoma of the skin and non-Hodgkin's lymphoma.

The metabolism of azathioprine leads to it being converted to 6-thioguanine. This is an analog for the DNA base 'guanine', leading to the incorporation of 6-thioguanine into replicating DNA. The thiol group is subject to chemical methylation. This results in the insertion of a modified base-pair (*S*-methylthioguanine) into the DNA strand. While the insertion is not frequent, it is highly miscoded during replication. This mechanism may be involved with carcinogenesis.

The effect of azathioprine on chromosomal aberration, micronuclei and sister chromatid levels is conflicted. But, there is evidence for an adverse effect in lymphocytes.

Azathioprine has a distinct mechanism of action in immunosuppressed patients who develop lymphoproliferative cancer that is strongly linked to Epstein-Barr infection. The immunosuppressive effect of azathioprine increases the risk of EBV infection. The carcinogenic mechanism of EBV is discussed in a later section of this chapter.

Chlornaphazine (Volume 4 & 42, Supplement 7)

Chlornaphazine is a bifunctional alkylating agent with mutagenic/genotoxic activity. In addition, the presence of sulfate esters of 2-naphthylamine as intermediates in the metabolism of chlornaphazine in rats is consistent with the production of 2-naphthylamine, and the increased incidence of bladder tumours in humans. (Chlornaphazine, IARC Volume 100A, p333)

Chlornaphazine (N,N-Bis(2-chloroethyl)-2-naphthylamine))is an antineoplastic agent that was used to treat Hodgkin's lymphoma. It is no longer in clinical use. It has been subject to limited research. One small epidemiological study linked it to bladder cancer. IARC concluded that chlornaphazine causes bladder cancer.

Chlornaphazine, as a bifunctional alkylating agent, likely shares etiological mechanisms with other members of this class. The limited evidence available shows evidence of genotoxic effects. *In vitro* studies have shown an induction of chromosomal aberrations in Chinese hamster cells and micronuclei in the bone-marrow cells of mice and rats, production of mutations in mouse lymphoma cells, and unscheduled DNA synthesis in rat hepatocytes.

Ciclosporin (also: Cyclosporine) (Volume 50)

'Ciclosporin is an immunosuppressant and long-term immunosuppression is linked to an increased risk of cancer. There are at least two facets to this. First, immunosuppression per se is associated

with cancer(s)..., These generally have a viral aetiology. ... In addition to these malignancies that usually arise early after immunosuppression is initiated, there are late effects – such as the development of skin cancer – that may have a different aetiology that could reflect direct or indirect effects of ciclosporin on DNA.' (Ciclosporin, IARC Volume 100A, p 343)

Ciclosporin is a potent immunosuppressive chemical that is used in patients undergoing organ transplantation. It has been causally associated with several cancers, most of which have a viral aetiology (e.g. Kaposi's sarcoma, cervical cancer and non-Hodgkin's lymphoma).

The immunosuppressive activity of ciclosporin is consistent with an increased risk for cancer due to impaired immune surveillance.

Ciclosporin induces increased synthesis of TGF-β and the consequent activation of its dependent transcriptional activators. Studies in cultured human pulmonary adenocarcinoma cells show evidence of a metaplastic phenotype. It is not clear if this effect contributes to the carcinogenicity of ciclosporin.

There is little evidence that ciclosporin directly damages DNA. One study reported an increased incidence of chromosomal aberrations in the lymphocytes of kidney transplant patients. A second study reported increased sister chromatid exchange in human peripheral blood lymphocytes.

There is evidence that ciclosporin can increase the level of double-strand DNA breaks. Ciclosporin induces oxidative stress in cells, leading to an increase in reactive oxygen species (ROS). The increased ROS levels lead to higher rates of single-strand DNA breaks, which are converted to double-strand breaks during DNA replication. This could lead to a chronic excess of double-strand breaks, producing a carcinogenic potential.

There have been several claims that ciclosporin inhibits the repair of ultraviolet induced DNA damage.

Plants Containing Aristolochic Acids (Volume 82)

'Key steps in the mechanism by which aristolochic acid causes tumours in experimental animals have been identified, and are consistent with events occurring in patients with urothelial cancers associated with aristolochic acid nephropathy and Balkan endemic nephropathy. The same DNA adducts identified in humans are also found in experimental animals....' (Plants Containing Aristolochic acids, IARC Volume 100A, p 359)

The term 'aristolochic acids' refers to an extract of Aristolochia species comprising a mixture of aristolochic acid I and its demethylated derivative, aristolochic acid II. These plants are used in traditional Chinese medicine. Plants containing aristolochic acid cause cancer of the renal pelvis and the ureter.

The carcinogenic mechanisms associated with these plants relate to the mechanism of action of aristolochic acid. These mechanisms are discussed in the next section about Aristolochic acid.

Aristolochic Acid (Volume 82)

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High doses of aristolochic acids cause severe necrosis of renal tubules, splenic and thymic atrophy and ulceration of the forestomach in animals.

Aristolochic acids are consistently active in *in vivo* and *in vitro* genotoxicity tests. The metabolism of aristolochic acids leads to the production of electrophilic cyclic N-acylnitrenium ions which react with DNA to produce adducts. These adducts have been identified and detected in exposed experimental animals and in urothelial tissues from patients with nephropathy subsequent to aristolochic acid use. The adducts lead to mutations which can activate oncogenes or inactivate tumour suppressor genes (e.g. *p53* or *RAS*). In rodent tumours, research has found activation of *RAS* oncogenes through a specific CAA→CTA transversion mutation in codon 61. In one nephropathy patient, a similar mutation was found in codon 139 of exon 5.

Updated Information

Aristolochic acid Aristolochic acid can induce apoptosis of human umbilical vein endothelial cells in vitro [22].

Methoxsalen plus Ultraviolet A Radiation (Supplement 7)

'Methoxsalen in combination with UVA is carcinogenic via a genotoxic mechanism that involves photo-activation' (Methoxsalen plus Ultraviolet A Radiation, IARC Volume 100A, p 372)

Methoxsalen (8-Methoxypsoralen) is a drug derived from plants which functions as a psoralen. In PUVA therapy, it is used in combination with ultraviolet light as a photosensitizing agent for the treatment of psoriasis and other skin lesions. Treatment requires activation of the psoralen with high-intensity long-wave length ultraviolet light (UVA). Carcinogenic effects also require UVA activation. Hence, mechanistic information needs to relate to the combination of the drug and UVA exposure. Methoxsalen, in combination with UVA radiation, causes squamous cell carcinoma of the skin.

PUVA has been shown to produce DNA adducts and other forms of DNA damage in a range of prokaryotic and eukaryotic cells. Methoxsalen is preferentially intercalated into DNA at 5'-TpA sites. Exposure to UVA leads to DNA alkylation. PUVA exposed Chinese hamster ovary cells revealed bi-adducts that are likely to be the major PUVA-induced pre-mutagenic lesions in mammalian cells.

In vitro studies of human cells found the induction of chromosomal aberrations, sister chromatid exchange, mutations, DNA damage, and DNA crosslinks. Similar results, with the addition of unscheduled DNA synthesis, were found in rodent cells grown in culture. PUVA can transform mouse C3H10T1/2 cells. Mitotic recombination and mutation were found in fungi, and mutation and DNA damage in bacteria.

Evidence suggests that PUVA treatment produces reactive oxygen species (including singlet oxygen and superoxide) that may have a role in PUVA-induced cytotoxicity.

Analgesic Mixtures Containing Phenacetin (Volume 13 & 24; Supplement 7)

'While there is evidence of genetic damage caused by phenacetin in various experimental systems, similar data are not available in humans'. (Phenacetin, IARC Volume 100A, p 395)

The carcinogenicity of analgesic mixtures containing phenacetin is related to the phenacetin component. The carcinogenic mechanisms of phenacetin are discussed on the next section.

Phenacetin (Volume 13 & 24; Supplement 7)

'While there is evidence of genetic damage caused by phenacetin in various experimental systems, similar data are not available in humans'. (Phenacetin, IARC Volume 100A, p 395)

Phenacetin (N-(4-Ethoxyphenyl)acetamide) is an analgesic that was available for use from 1887 but was withdrawn in the mid-1980's due to an elevated risk of kidney disease. Phenacetin causes cancer of the renal pelvis and ureter.

Evidence for a carcinogenic mechanism for phenacetin is conflicting. There are no studies showing genetic or other effects in humans.

Recent animal and cell culture studies have found evidence of an increased frequency of micronuclei in mice and rat bone marrow cells. *In vitro* studies induced chromosomal aberrations in Chinese Hamster cells and DNA strand breaks in rat and human bladder cells. One study reported induction of micronuclei, but only by a metabolite of phenacetin, not by the drug itself. In contrast, older studies revealed equivocal evidence on the induction of chromosomal aberrations, sister chromatid exchange and micronuclei in rodents treated with phenacetin.

Phenacetin shows mutagenicity when tested in bacterial systems in the presence of a metabolic system derived from hamster or rat liver. No mutagenicity has been seen when testing used a metabolic system from a mouse. Oral feeding of phenacetin to mice with a deficiency in nucleotide-excision repair produced an increased mutation frequency in a Lac Z reporter gene in the kidney.

Phenacetin induces cell proliferation in the urothelium of the kidney, the bladder, the renal pelvis, and DNA synthesis in the nasal respiratory and olfactory mucosa of rats.

Volume 100B Biological agents

There is a very extensive literature concerning the molecular actions of the biological agents discussed in Volume 100B. Many of the molecular processes underlying the infection process by a biological agent and the subsequent growth of the agent in the body are relevant to potential carcinogenic mechanisms. It is not feasible to precise all of the relevant material in the brief summaries being presented in this chapter. Instead, a synopsis will be presented of the key processes. Interested readers are referred to Volume 100B and its associated cited material for more detail.

Epstein-Barr Virus (Volume 70)

'Mechanistic data that strongly support an oncogenic role of EBV in human cancer can be summarized as follows:

- *EBV immortalizes normal B cells in culture.*
- *One or several EBV gene products are expressed in all EBV-associated cancers.*
- *At the molecular level, these EBV-encoded gene products associated with latent viral infection induce cell proliferation, block apoptosis, induce genomic instability or modulate cell migration. These events occur before or during tumour initiation. Several of these gene products are also involved in mechanisms contributing to continued tumour maintenance, cell growth, and progression.' (Epstein-Barr virus, IARC Volume 100B, p 80)*

The Epstein-Barr virus (EBV) is a ubiquitous virus that has infected up to 95% of people by the time they reach adulthood. Once the initial infection is controlled by the immune system, EBV persists in a latent state inside B cells of the immune system. Evidence suggests that re-activation by an external agent is required to trigger carcinogenic potential. Activation agents can include: infections (especially malaria in relationship to Burkitt's lymphoma), immunodeficiency and potentially food (e.g. salted fish in China) and chemical exposures. EBV causes several types of lymphoma and cancer of the nasopharynx.

EBV expresses six latent nuclear proteins and three latent membrane proteins. All of these have been shown be multi-functional and to affect cellular signalling pathways in ways which could contribute to tumorigenesis. In addition, EBV expresses two non-coding RNAs that contribute to B-Cell transformation and over 22 miRNAs that target genes that are relevant to carcinogenesis.

The IARC Volume 100B summarizes the mechanistic evidence for EBV-associated oncogenesis as follows (quoted intact):

- The ability of EBV to immortalize human B lymphocytes *in vitro*
- Other effects of EBV infection of human cells *in vitro* affecting their phenotype – migration and invasion
- Convincing links of these phenotypic effects on cell proliferation, apoptosis, and cell migration to single EBV proteins or combinations thereof, primarily by the expression of, or "knock down" of, single proteins.
- Induction of EBV-positive lymphoproliferative diseases or lymphomas by infection of animals (New World monkeys) with EBV, or transplantation of infected human B lymphocytes to immunosuppressed mice (SCID or nude).

- At the molecular level, these EBV-encoded gene products associated with genomic instability or modulate cell migration. These events occur before or during tumour initiation. Several of these gene products are also involved in mechanisms contributing to continued tumour maintenance.

Updated Information

A study showed that the EBV nuclear antigens EBNA-1 and EBNA-3C, and the latent membrane protein LMP-1, independently promote genomic instability, as detected by nonclonal chromosomal aberrations, DNA breaks and phosphorylation of histone H2AX [23]. EBV was also able to induce oxidative stress in human B lymphocytes, epithelial, and lymphoblastoid cell lines *in vitro* [24,25], and to increase the levels of micronuclei in human cells *in vitro* [26,27]. Altered DNA methylation [28,29] and down-regulation of micro-RNAs [30] were reported in gastric carcinomas associated with EBV infection. EBV altered DNA methylation patterns *in vitro* [31,32] and induced histone modifications [33,34]. Furthermore, EBV-encoded LMP-1 induced MicroRNA-10b [35,36].

EBV has immune effects as evidenced by the ability of the virus to induce the release of chemotactic cytokines or chemokines in human neutrophils (accumulation of mRNA for IL-8 and macrophage inflammatory protein-1 alpha (MIP-1 alpha) [37,38]. Furthermore, EBV antigens could generate suppressor cell activity in vitro [39].

Chronic Infection with Hepatitis B Virus (Volume 59)

'There is strong evidence to support an indirect role for HBV in hepatocarcinogenesis resulting from chronic necro-inflammatory hepatic disease (cirrhosis), as well as moderate evidence for a direct role largely associated with HBx (a protein transcribed from the HBV genome).' (Hepatitis B virus, IARC Volume 100B, p 123)

The Hepatitis B virus (HBV) is a common DNA virus that displays strong geographic variability. It causes both acute and chronic hepatitis, and can be present in an inactive carrier state. It is established as a causal agent for liver cirrhosis and hepatocellular carcinoma (HCC). Hepatitis and cirrhosis arise as a result of the body's immune system response to the virus and infected cells. HBV causes hepatocellular carcinoma (HCC).

'At a molecular level, the genesis of HBV-induced HCC is a complex, multifaceted, and multistep process with the essential components being a series of genetic or epigenetic changes in the genes that govern cell proliferation and cell death.' (IARC Volume 100B, p 113).

A large proportion of HCC arises in the presence of chronic hepatitis or cirrhosis, suggesting that a chronic necro-inflammatory process contributes to cancer development. This process is outlined in Figure 4.1 (p 116) of IARC Volume 100B. There is evidence to suggest that this process leads to the generation of reactive oxygen or nitrogen species that cause DNA damage.

HBV can integrate into the cellular genome. While not required for virogenesis, it has been found in over 85% of HBV-related HCC's. Increased cellular proliferation from an inflammatory response can produce double-strand DNA breaks and facilitate viral integration. Integration occurs at random throughout the DNA but integration near a variety of genes associated with cellular division and stability has been noted.

HBx functions through protein-protein interactions. HBx activates transcription of a wide variety of proteins involved with regulation of cellular function, including p53. A wide variety of cis-elements are responsive to HBx, including many transcription factors. HBx directly interferes with DNA repair by forming a complex with the DNA-repair protein XAP-1.

Methylation and epigenetic gene silencing are important mechanisms in the early stages of for HCC. HBx may contribute to this process by deregulating expression of DNA methyltranferases.

Updated Information

Recent studies have documented effects of HBx on several factors that may have impact on cancer risk, including c-myc, p53, p21 and miRNAs [40,41,42,43,44]. The impact on histone functioning through the induction of metastasis-associated protein 1 (MTA1) and histone deacetylase (HDAC) has been confirmed [45]. Differential expression of 188 miRNAs has been noted both *in vitro* [42] and *in vivo* [44].

HBV-infected patients diagnosed with chronic hepatitis, cirrhosis and hepatocellular carcinoma have a 20-90-fold increased urinary level of Etheno-deoxyadenosine adducts [46], supporting the presence of oxidative stress and chronic inflammation.

HBV proteins have been shown to induce apoptosis in humans and animals *in vivo* and *in vitro* [47,48,49,50], and to promote cellular proliferation and differentiation in human cells [49,51] and also in animal cells [52,53].

Chronic Infection with Hepatitis C Virus (Volume 59)

'Although there is strong evidence that HCV is one of the leading causes of HCC [hepatocellular carcinoma], there is still much to understand regarding the mechanism of HCV-induced transformation. While liver fibrosis resulting from long-lasting chronic inflammation and liver regeneration resulting from immune-mediated cell death are likely factors contributing to the development of HCC, the direct role of HCV proteins remains to be determined. Many in vitro studies have shown that HCV expression may interfere with cellular functions that are important for cell differentiation and cell growth.' (Hepatitis C virus, IARC Volume 100B, p 158)

Hepatitis C virus (HCV) is an RNA virus that is endemic around the world. It is largely transmitted via blood and has a high prevalence among intravenous drug users, especially where needle sharing occurs. Acute infection leads to persistent infection in up to 90% of cases. HCV causes hepatocellular carcinoma and non-Hodgkin's lymphoma.

HCV replicates exclusively within the cytoplasm of a cell. Therefore, it does not directly interact with DNA; rather, potentially pro-carcinogenic events are restricted to the cytoplasm. HCV replication is directly linked to the endoplasmic reticulum and lipid metabolism. The proteins expressed by HCV interact with cellular components.

Chronic inflammation and endoplasmic reticulum stress lead to oxidative stress and disruption of the intracellular redox state that leads to genomic damage. Some of the HCV proteins interact directly with cellular signalling cascades and affect cell metabolism and replication.

HCV causes steatosis (through impairing lipid excretion and metabolism and enhancing lipid genesis in the liver). The carcinogenic effect is enhanced through a positive feedback loop involving steatosis, insulin resistance and endoplasmic reticulum stress.

Updated Information

N-Ras expression was increased in the detergent-resistant fractions from HCV genomic replicon clones compared to control cells [54]. HCV also interferes with the epigenetic apparatus of the cell by changing DNA methylation in humans [55,56,57], inducing histone modifications in human cells *in vitro* [58,59], and deregulation of micro RNA levels in human cells [42,60,61,62].

HCV interferes with the pathway controlling apoptosis [63] but the impact on cellular proliferation is unclear [63,64]. HCV can interfere with DNA damage repair [65,66,67]. Elevation of telomerase activity has also been observed [68].

Kaposi Sarcoma Herpes Virus (Volume 70)

'At the molecular level, KSHV-encoded gene products associated with latent viral infection induce cell proliferation, block apoptosis, induce genomic instability or modulate cell migration and tumour progression.' (Kaposi Sarcoma Herpes Virus, IARC Volume 100B, p195)

Kaposi Sarcoma Herpes Virus (KSHV) is a DNA virus and a member of the herpes virus family. It is able to produce life-long latent infections; CD19-positive B-cells are a major reservoir. It is mainly transmitted via saliva and has a peak age of infection between age 6 and 10 in high prevalence countries. It causes Kaposi's sarcoma.

Infection with KSHV converts primary endothelial cells into spindle cells. KSHV proteins have been shown to interfere with apoptosis. There is little evidence to suggest that KSHV produces DNA damage or genetic instability. Five KSHV proteins have been shown to have cell transformative properties *in vitro*. An additional three proteins affect cell-cell regulation and tumour cell survival. KSHV produces many changes to cellular gene expression and transcription. These tend to stimulate cellular proliferation and change differentiation potential.

Updated Information

Natural killer cells from subjects infected with KSHV (both asymptomatic and those who developed Kaposi sarcoma) exhibited changes in expression of several genes including CD161 receptors [69]. miRNAs of KSHV induce B-cell proliferation [70]. KSHV induces cell proliferation and differentiation in vivo and in vitro [69,71].

Infection with Human Immunodeficiency Virus-1 (Volume 67)

'HIV-1 increases the cancer risk in humans indirectly, primarily by immunosuppression. .... Suggested mechanisms include HIV-1-mediated immune dysregulation, in particular B-cell hyper-activation, and perhaps effects of the secreted HIV-1 Tat protein. However, unlike what is known about other cancer-associated viruses, there is no evidence that HIV-1-infection by itself leads to cell transformation or immortalization.' (Human immunodeficiency virus-1, IARC Volume 100B, p 240)

Human immunodeficiency virus-1 (HIV-1) is the causative agent for AIDS. It is an RNA virus that transcribes its RNA core into DNA (through the action of a reverse-transcriptase), which is then integrated in the host cell DNA. It primarily infects CD4+ T-cells, macrophages and dendritic cells.

There is no evidence that HIV-1 directly causes cancer. Rather, it increases cancer risk through the severe immunodeficiency it produces, leading to an increase in cancer risk from secondary carcinogenic agents. For example, non-Hodgkin Lymphoma risk is increased as a result of '*the*

profound depletion of CD4-positive T lymphocytes that is caused by HIV-1 [and] allows the dysregulation of control of B cells, and the expression of the effects of lymphotrophic viruses' (IARC Volume 100b, p223). Despite the integration of the cDNA transcript of the viral RNA, there is no evidence that HIV causes chromosomal or genetic damage.

Human Papilloma Virus Types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 (Volumes 64, 66, & 90)

'The [carcinogenic] mechanisms involve immortalization, transformation, inhibition of apoptosis, induction of genomic instability, and deregulation of the immune response. The E6 and E7 genes of HPV16 and HPV18 have been the most extensively studied and were found to confer a similar set of biological phenotypes (e.g. immortalization, inhibition of DNA-damage response, genomic instability, inhibition of differentiation). The E6 .... protein ... [produces] inactivation of p53, induction of hTERT, binding to PDZ; ... E7[produces] inactivation of pRb and related pocket proteins, activation of E2Fs.' (Human Papillomaviruses, IARC Volume 100B, p294-5).

The human papilloma virus (HPV) family consists of DNA viruses and has over 100 types with 4 main genus groups. They most commonly infect mucosal tissue (the alpha genus) or cutaneous tissue (the beta genus). HPV displays long persistence without overt signs of infection. HPV causes cervical cancer.

Carcinogenic potential varies by type, with HPV-16 and HPV-18 having the strongest connection to cervical cancer aetiology. DNA transcription of the viral DNA produces seven proteins of which two (E6 and E7) are most strongly associated with carcinogenesis.

The E6 and E7 proteins of the alpha genus of HPV target cellular proteins for degradation through the ubiquitin proteasome pathway. Multiple target proteins have been identified. Of particular relevance for E6 is the P53 protein. E7 targets pRb, p107, p130 and related pocket proteins. E6 also enhances telomerase activity through an unknown mechanism. E7 alters transcriptional regulations through AP1 transcription factors, histone deacetylases and MPP2.

HPV can compromise the functioning the normal DNA-repair processes and cellular response to DNA damage. This leads to genetic instability and chromosomal abnormalities. E7 stimulates cellular proliferation while E6 inhibits apoptosis. Persistence of viral infection and expression of E6/7 is required for carcinogenesis, suggesting that the virus might alter immune system response.

Updated Information

HPV interferes with epigenetics of the cell by changing DNA methylation [72,73,74], inducing histone modifications [75,76] and modulation of micro RNAs expression [77,78]. It also changes the expression of monocyte chemoattractant protein 1 (MCP-1) which plays an important role in the recruitment of monocytes in solid tumours [79,80] and impairs DNA repair [81,82]. HPV can induce immortalization of human cells *in vitro* [83,84].

Human T-cell Lymphotropic Virus Type 1 (volume 67)

'There is strong mechanistic evidence supporting the role of HTLV-1 in human carcinogenesis. The viral protein Tax has the ability to immortalize and to transform human T cells. At the leukemic stage, the expression of Tax is often not maintained, but the viral protein HBZ continues to be

expressed, and supports the sustained growth of the leukemic cells.' (Human T-cell Lymphotropic Virus Type 1, IARC Volume 100B, p 332).

Human T-cell Lymphotropic Virus Type 1 (HTLV-1) is a complex retrovirus that contains two copies of its genomic RNA strands. It can infect multiple cell types but only induces transformation in T-lymphocytes. It causes adult T-cell lymphoma/leukemia.

HTLV-1 expresses six proteins of which *tax* is essential for carcinogenesis. *Tax* has been shown to activate and repress multiple genes, modulate the cell-cycle and repress apoptosis. Transcription pathways activated by HTLV-1 include: NF-κB, CREB, SRF, myc and AP-1. It inhibits the function of *p53*. There is associated genomic instability and the development of aneuploidy. Epigenetic silencing has been noted around a common breakpoint region in chromosome 10p11.2. Long-term viral persistence is required for carcinogenesis, suggesting that immune system abnormalities may be important.

Updated Information

HTLV-1 increases the frequency of micronuclei [85] and induces chromosomal aberrations in humans [86,87]. Epigenetic changes have now been shown to induce histone modifications [88,89] and dysregulation of micro RNAs [90,91,92]. IHYLV-1 can induce angiogenesis by establishing gap junction intercellular communication with endothelial cells [93,94]. A bystander effect has been observed in HTLV-1 induced carcinogenesis [95,96].

Chronic Infection with Opisthorchis Viverrini (Volume 61)

'Liver-fluke-induced cholangiocarcinoma is likely the result of chronic inflammation, involving the activation of oxidative stress pathways. Metabolic products excreted from liver flukes are highly immunogenic and may stimulate cell proliferation and anti-apoptosis directly.' (Adapted from: Opisthorchis viverrini and Clonorchis sinensis, IARC Volume 100B, p 365)

Opisthorchis viverrini and *Clonorchis sinensis* are liver flukes that are endemic in Asia, particularly China, Thailand, Vietnam, Laos and the Republic of Korea. Their lifecycles involve snails and fish as intermediary hosts. Human infection is largely from eating raw or undercooked fish. Both of these liver flukes have been identified as causal agents for cholangiocarcinoma.

Liver flukes produce histopathological changes characterized by inflammation, hyperplasia and metaplasia, with a high frequency of changes in the bile duct. They excrete various metabolic products that are highly immunogenic. These increase cellular proliferation through stimulation of pRB and cyclin D1. The immune response leads to the production of NDMA and nitric oxide (NO) and to the nitrosation of amines. Liver fluke infection is linked to diffuse nitrosative and oxidative DNA damage and adduct formation. There is little evidence to support any direct genetic or epigenetic effects.

Updated Information

Expression of c-Ski, TGF-β and Smad4, were greatly up-regulated [97] in a hamster model, and in humans. Furthermore, mutations of Kras and TP53 were found in a small proportion of *Opisthorchis viverrini*-associated cholangiocarcinomas in a hamster model [98]. There was increased expression of proteins related to stress response, DNA replication and repair, and cell structure [99]. Increased expression of DNA repair enzymes has been noted [100].

Chronic Infection with Clonorchis Sinensis (Volume 61)

'Liver-fluke-induced cholangiocarcinoma is likely the result of chronic inflammation, involving the activation of oxidative stress pathways. Metabolic products excreted from liver flukes are highly immunogenic and may stimulate cell proliferation and anti-apoptosis directly.' (Adapted from: Opisthorchis viverrini and Clonorchis sinensis, IARC Volume 100B, p 365)

The two liver flukes classed as Group 1 Human Carcinogens *(Opisthorchis viverrini* and *Clonorchis sinensis)* have similar mechanisms of action. These are described in the previous section for *Opisthorchis viverrini.*

Chronic Infection with Schistosoma Haematobium (Volume 61)

'S. haematobium with egg deposition in the tissue leads to severe inflammation of the urinary bladder wall .... resulting in increased oxidative stress. ...[T]he observed increased levels of oxidative stress .... point towards a relationship between oxidative stress induced by continuous and chronic inflammation due to schistosome infection and possibly nitric-oxide-mediated DNA genotoxicity .... and alkylation of DNA by N-nitroso compounds.' (Schistosoma haematobium, IARC Volume 100B, p 382)

Schistosoma haematobium is a parasite endemic to tropical regions in Africa and the Middle East that reproduces in a human host. Eggs are excreted in urine where they pass through a fresh-water snail with the human host becoming infected by contact with water where the snails live. The adult worm attaches in venous blood vessels surrounding the bladder; eggs are released through the bladder wall. About 50% of eggs remain in the bladder wall, provoking an inflammatory response. *Schistosoma haematobium* causes squamous cell bladder carcinoma.

'Several studies indicate that the carcinogenicity of S. haematobium is a multifactorial and multistage process where several mechanisms are involved. S. haematobium eggs induce a chronic inflammation and irritation in the urinary bladder. The inflammatory response around the eggs gives rise to genotoxic factors and products that may cause genomic instabilities of host cells, leading to modifications in the regulation of tumour-suppressor genes and oncogenes as well as stimulating a proliferative response of the host cells to repair tissue damage caused by the inflammation.' (IARC Volume 100B, p 378).

There is evidence for DNA adduct production and an increase in gene methylation. Oxidative stress markers are also elevated.

Updated Information

Chinese Hamster ovary cells treated in culture with *S. haematobium* total antigen showed increased proliferation, increased S-phase and decreased apoptosis, as well as down-regulation of tumour suppressor p27 and up-regulation of anti-apoptotic molecule Bcl-2 [101] and down-regulates the transcriptional activity of oestrogen receptor in HCV29 cells and mice [102].

Chronic Infection with Helicobacter Pylori (Volume 61)

'Multiple lines of evidence point to a central role for the chronic gastric inflammatory response and resulting oxidative stress in H. pylori-associated gastric carcinogenesis. This leads to altered cellular turnover accompanied by changes in gene expression, methylation, and mutation' (Helicobacter Pylori, IARC Volume 100B, p 422)

Helicobacter Pylori is a gram-negative bacterium that exists mainly in mucous-secreting gastric cells. It causes non-cardia gastric carcinoma and MALT leukemia. There is evidence that chronic *H. pylori* infection reduces the risk of adenocarcinoma of the oesophagus, perhaps through the production of gastric atrophy.

Non-cardia gastric carcinomas normally arise in areas of chronic inflammation. *H. Pylori* is the primary cause of gastritis. An especially intense inflammatory response to *H. pylori* is thought to induce greater gastric epithelial cell damage, faster cell turnover, and the eventual emergence of gastric epithelial cells carrying cancer-prone mutations. Several reports have demonstrated that *H. pylori* alters the expression of specific oncogenes and tumour suppressor genes implicated in gastric carcinogenesis. *H. pylori* infection promotes the nuclear translocation of β-catenin, thereby activating downstream β-catenin-responsive genes including cyclin D. It up-regulates the p53 homologue p73 in gastric cells, which leads to promotion of apoptosis and decreases expression of the cell cycle inhibitory protein p27 that is known to be lost in aggressive gastric cancers.

Updated Information

H. pylori induces the formation of DNA strand breaks in humans [103,104,105,106] and the formation of micronuclei in humans [107,108]. It has also been shown to alter DNA methylation [109,110,111], induce histone modifications [112,113], change expression patterns of miRNAs [114] and induce changes in gene expression [115,116,117]. *H. pylori* can interfere with intercellular gap communications [118,119].

VOLUME 100C: ARSENIC METALS, FIBERS, DUST

Arsenic and Inorganic Arsenic Compounds (Volume 1, 2 & 23, Supplement 7)

'...the evidence points to weak or non-existent direct mutagenesis, which is seen only at highly cytotoxic concentrations. On the other hand, long term, low-dose exposure to inorganic arsenic .... is likely to cause increased mutagenesis as a secondary effect of genomic instability, perhaps mediated by increased levels of reactive oxygen species, as well as co-mutagenesis with other agents. The major underlying mechanisms observed at low concentrations include the rapid induction of oxidative DNA damage and DNA-repair inhibition, and slower changes in DNA-methylation patterns, aneuploidy, and gene amplification.' (Arsenic and Arsenic Compounds, IARC Volume 100C, p 84 [3])

Arsenic is a common element that is widely present in the environment as well as having a major role in manufacturing and in a wide range of chemicals. The main route of human exposure is via ingestion, with very high levels in drinking water providing a major route of exposure in some regions. Chronic arsenic exposure is a cause of lung cancer, bladder cancer and skin cancer.

Arsenicals do not react directly with DNA. The major underlying carcinogenic mechanisms observed at low concentrations include the rapid induction of oxidative DNA damage and DNA-repair inhibition. Some forms of arsenic can induce chromosomal aberrations *in vitro* but statistically significant increases in chromosomal aberrations occur only at toxic doses. Chronic low-dose exposure in animal models produces genomic instability and can lead to chromosomal aberrations. Production of micronuclei has been observed and As^{III} can interfere with spindle function during mitosis. Increased mutagenesis is observed as a consequence of increased genomic instability.

Global hypomethylation of DNA, combined with focal hypermethylation of selected genes, has been observed in animal models and humans. Gene amplification has been reported from some animal experiments. An adverse impact on DNA repair and *P53* function has been noted, although other studies reported increased DNA repair activity, perhaps related to antioxidant effects in response to arsenic induced oxidative damage. Arsenic can stimulate an inflammatory response and interfere with apoptosis with chronic exposure.

Updated Information

Arsenic can induce changes in gene expression. In human cell lines, arsenic induced genes related to cytotoxicity and cellular proliferation [120]. In rat kidney SC/PC cell line, chronic exposure to low doses of arsenite increased the secretion of matrix metalloproteinase (MMP), and Cox-2 expression and increased the cellular proliferation rate [121].

Beryllium and Beryllium Compounds (Volumes 1,23, 42 & 58; Supplement 7)

'....chromosomal aberrations and aneuploidy were observed in vivo in mice, at nontoxic concentrations. ....[B]eryllium is capable of producing oxidative stress .... [T]oxicity of beryllium in the lung may lead to cell killing and compensatory cell proliferation. .... [I]nflammatory processes induced by beryllium may also cause an increase in reactive oxygen species, mediate cell turnover, and alter cell-signalling pathways. Furthermore, down-regulation of genes involved in DNA synthesis, repair and recombination also occurs. Thus, the processes underlying beryllium induced

carcinogenesis are clearly complex, with several possible interactive mechanisms.' (Beryllium and Beryllium Compounds, IARC Volume 100C, p 116 [3])

Beryllium is an uncommon metal that is used primarily in alloys or beryllium oxide ceramics. Beryllium causes lung cancer in an occupational setting. Inhalation seems to be required for carcinogenesis; there is no evidence of a cancer risk from beryllium ingestion.

There is little evidence for direct genotoxicity although down-regulation of DNA synthesis and repair genes has been noted. In general, based on the IARC Volume 100C material, the carcinogenic mechanisms associated with Beryllium have not been extensively studied.

Beryllium is known to produce a chronic allergic-type lung response and disease called Berylliosis. The inflammatory response to this condition leads to enhanced cellular proliferation, oxidative stress and altered cellular signalling pathways. *In vitro* studies in mice reveal formation of reactive oxygen species, with marked increases in apoptosis and activation of caspase 8.

Updated Information

One study has shown that beryllium exposure induces I-CAM1 expression on the cell surface of small airway epithelial cells and enhanced the release of soluble I-CAM1 into the extracellular medium [122]. Another study found that, in microorganisms and in mammalian cells, soluble beryllium compounds can produce some infidelity of *in vitro* DNA synthesis and forward gene mutations [123]. Beryllium may also induce morphological transformation *in vitro* [124]. An *in vivo* study in mice suggested that beryllium induction of oxidative stress and unrepaired DNA damage may be due to down-regulation in the expression of DNA repair genes [125].

Cadmium and Cadmium Compounds (Volumes 2, 11, 42, & 58, Supplement 7)

'Several mechanisms have been identified that potentially contribute to cadmium-induced carcinogenesis. Direct binding to DNA appears to be of minor importance, and mutagenic responses are weak. Convincing evidence exists on disturbances of DNA-repair and tumour-suppressor proteins, which lead to chromosomal damage and genomic instability. Further reported effects include changes in DNA-methylation patterns as well as interactions with signal-transduction processes, which may contribute to the deregulation of cell growth.' (Cadmium and Cadmium Compounds, IARC Volume 100C, p 140 [3])

Cadmium is used widely as a key component in NI-CD batteries. Exposure of the general public is mainly through food ingestion and drinking water although smokers receive exposure from cigarettes. Occupational exposure is mainly through inhalation. Cadmium causes lung cancer.

Cadmium does not directly damage DNA when studied in cell extracts. However, *in vitro* studies in mammalian cells and rodents show that it causes double-strand DNA breaks, chromosomal aberrations and produces micronuclei. Cadmium is not mutagenic in bacteria. Cadmium increases the mutagenicity of ultraviolet radiation, alkylation, and oxidation in mammalian cells.

Cadmium induces oxidative stress in both *in vitro* and *in vivo* systems. Since cadmium is not redox-active, this effect is most likely due inhibition of antioxidant enzymes and DNA repair.

Cadmium inhibits several types of DNA-repair mechanisms. These effects may relate to the displacement of zinc from zinc finger structures.

Cadmium interacts with a multitude of cellular signal transduction pathways. *In vitro* studies in several cell types found that cadmium induces the receptor-mediated release of the second messengers inositol-1,4,5-trisphosphate and calcium, activates various mitogenic protein kinases, transcription and translation factors, and induces the expression of cellular proto-oncogenes such as *c-fos*, *c-myc*, and *c-jun*. Cadmium also inhibits the negative controls of cell proliferation. It inactivates the tumour suppressor protein p53, and inhibits the p53 response to damaged DNA and reduces DNA methylation.

Updated Information

Cadmium can affect the genes involved in growth regulation on initiated cells during the promotion stage of *in vitro* cell transformation [126]. Induction of DNA hypomethylation have been shown in humans *in vivo* and *in vitro* [127,128].

Chromium (VI) Compounds (Volumes 2, 23, 49, & 51, Supplement 7)

'Several mechanisms are involved in the carcinogenesis induced by chromium (VI) that include the induction of DNA damage, the generation of oxidative stress and aneuploidy, leading to cell transformation. With respect to DNA damage, the spectrum of induced lesions appears to depend strongly on the cellular reductant involved. Thus, under physiological conditions with ascorbate as the major reductant, the generation of pre-mutagenic ternary chromium–ascorbate–DNA adducts appears to be of major relevance, which may be linked to the increased number of mismatch-repair-resistant cells observed in chromate-induced lung tumours.' (Chromium (VI) Compounds, IARC Volume 100C, p 163 [3])

Chromium (VI) compounds involve chromium in an oxidative state that occurs mainly in manufactured products. It reduces to Chromium (III) in the presence of reducing agents (e.g. iron) or oxidizable organic matter. General population exposure to these compounds is by inhalation from anthropogenic sources or from drinking water. Occupational exposure is through inhalation or dermal contact. Most research on human cancer risk was conducted on occupational settings. Chromium (VI) compounds cause lung cancer.

Chromium (VI) compounds are genotoxic, both *in vitro* and *in vivo*. Workers exposed to chromium (VI) in dust displayed elevated levels of DNA strand breaks, sister chromatid exchanges and micronuclei. Dominant lethal mutations have been observed in male mice.

Chromium (VI) is reduced to Chromium (III). When this occurs extracellularly, the chromium III formed cannot penetrate the cell membrane. However, CrIII can also be formed through a series of intracellular processes, using ascorbate and cysteine as key reductants. This process leads to the formation of DNA adducts (mainly ternary adducts), DNA-DNA and DNA-protein cross-links, DNA strand breaks and oxidative DNA-base pair modifications. One model suggests that chronic chromium (VI) exposure leads to the outgrowth of mismatch-repair deficient clones.

The process of reduction of chromium (VI) can release potentially toxic intermediates such as hydroxyl radicals, superoxide and nitric oxide.

Nickel Compounds (Volumes 1,11,42,45,49 & 67, Supplement 7)

'The ultimate carcinogenic species in nickel carcinogenesis is the nickel ion Ni(II). .... Nickel compounds are not mutagenic in bacteria, and only weakly mutagenic in mammalian cells under standard test procedures, but can induce DNA damage, chromosomal aberrations, and micronuclei

in vitro and in vivo. .... Nickel compounds act as co-mutagens with a variety of DNA-damaging agents. Thus, disturbances of DNA repair appear to be important. A further important mechanism is the occurrence of epigenetic changes, mediated by altered DNA methylation patterns, and histone modification. Inflammation may also contribute to nickel-induced carcinogenesis.' (Nickel and Nickel Compounds, IARC Volume 100C, p210)

Nickel is a metal that is widely used in manufacturing and industrial processes. It is present in a wide range of alloys and compounds such as oxides, sulfides and salts. The general population is largely exposed through ingestion of food. Occupational exposure is mainly via inhalation. Nickel causes lung cancer, and cancers of the nasal cavity and paranasal sinuses.

The ultimate carcinogenic/genotoxic species is Nickel (II). However, Nickel (II) does not react directly with DNA. *'Genotoxic effects have been consistently observed in exposed humans, in experimental animals, and in cell culture systems, and include oxidative DNA damage, chromosomal damage, and weak mutagenicity in mammalian cells.*' (IARC Volume 100C, p 208) Various genotoxic effects (e.g. sister chromatid exchange, chromosomal aberrations, micronuclei) have been observed but only at toxic levels of nickel. It is hypothesized that the observed genotoxic effects are largely secondary to inflammation and oxidative stress.

Nickel (a redox-active metal) has been shown to increase reactive oxygen species in many cell types. This may involve catalyzing Fenton-type reactions. Nickel inhibits nucleotide-excision and base-excision DNA repair. Specific inhabitation of XPA and 3-methyladenine-DNA glycosylase II has been demonstrated. Nickel compounds are able to cause gene silencing with genes located near heterochromatin being specific targets. Nickel also causes ubiquination and phosphorylation of histones.

Updated Information

Crystalline nickel sulfide has been found to induce genomic instability in transformed 16 human broncho-epithelial cells [129].

All Forms of Asbestos (Chrysotile, Amosite, Crocidolite, Tremolite, Actinolite, and Anthophyllite) (Volumes 2,14,42, & 45, Supplement 7)

'The mechanistic basis for asbestos carcinogenicity is a complex interaction between crystalline mineral fibres and target cells in vivo. .... The following general mechanisms have been proposed for the carcinogenicity of asbestos fibre..... Asbestos and erionite fibres have been shown to generate free radicals that directly induce genotoxicity as assessed by DNA breaks and oxidized bases in DNA. Asbestos fibres have also been shown to interfere with the mitotic apparatus by direct physical interaction resulting in aneuploidy and polyploidy. ... asbestos fibres have been shown to induce macrophage activation and persistent inflammation that generate reactive oxygen and nitrogen species contributing to tissue injury, genotoxicity, and epigenetic alterations. ....[These are] associated with the activation of intracellular signalling pathways, resistance to apoptosis, and stimulation of cell proliferation.' (Asbestos (Chrysotile, Amosite, Crocidolite, Tremolite, Actinolite, and Anthophyllite), IARC Volume 100C, p 294 [3])

Asbestos is a generic designation for a range of naturally occurring mineral silicate fibres with six main varieties. It has been extensively studied, both epidemiologically and mechanistically, as shown by the 75 page discussion in IARC Volume 100C. The primary route of exposure is inhalation although ingestion from drinking water is a secondary source. Workers can exposure

family members through fibres attached to their clothing. All forms of asbestos cause mesothelioma and a range of other cancers including: lung, larynx, and ovary. Asbestos is also likely a cause of cancers of the pharynx, stomach and colorectum.

The carcinogenic mechanism is complex and involves interactions between the fibres and target cells (an overview is presented in figure 4.2, IARC Volume 100C, p 289). The major carcinogenic mechanism involves an inflammatory response to the fibres (which can lead to chronic lung fibrosis (asbestosis)). Alveolar macrophages phagocytize the fibres, leading to the release of cytokines, growth factors, oxidants, etc. (see Figure 4.1, IARC Volume 100C, p 284). Phagocytosis can be incomplete or fail, which can lead to macrophage apoptosis. This response can generate an excess of reactive oxygen and nitrogen species. This initiates a wide range of down-stream genetic and epigenetic events leading to DNA damage and carcinogenesis. In summary, the inhalation of asbestos fibres is associated with excess generation of reactive oxygen and nitrogen metabolites, cell injury, apoptosis, and persistent lung inflammation.

Asbestos fibres can also physically interfere with the mitotic machinery in cells.

Erionite (Volume 42, Supplement 7)

'See Section ... on Asbestos' (Erionite, Volume 100C, p 315 [3]).

Erionite is a naturally occurring fibrous mineral that belongs to a group of hydrated aluminosilicate minerals called zeolites. It causes mesothelioma.

The carcinogenic mechanism is similar to that of Asbestos and will not be discussed further.

Leather Dust (Volume 25, Supplement 7)

See Section ... on Wood Dust (Leather Dust, Volume 100C, p 350 [3]).

The term 'Leather Dust' is used to include a range of exposures including: leather goods manufacturing, leather industries, and leather tanning/processing. These had been examined in separate sections in previous IARC reviews but are combined as one section in Volume 100C.

Leather is produced from the skin or hide of animals through a tanning process using either vegetable tannins or chromium (III) sulphate. Leather is frequently treated with chemicals such as benzidine-dyes and chemical solvents (e.g. toluene, benzene, acrylic resins, polyurethane). Leather dust is created from the working of leather throughout the tanning and manufacturing process, leading to a very complex and variable exposure. Leather dust causes nasal and paranasal sinus cancers, most commonly of the adenocarcinoma type.

Carcinogenic mechanisms are discussed in the section on Wood Dust.

Crystalline Silica in the Form of Quartz or Cristobalite Dust (Volumes 42, 68 & 81, Supplement 7)

'Three mechanisms have been proposed for the carcinogenicity of crystalline silica in rats.... First, exposure to crystalline silica impairs alveolar-macrophage-mediated particle clearance....which results in macrophage activation, and the sustained release of chemokines and cytokines.....that induce genotoxicity, injury, and proliferation of lung epithelial cells leading to the development of lung cancer. Second, extracellular generation of free radicals by crystalline silica depletes antioxidants in the lung-lining fluid, and induces epithelial cell injury followed by epithelial cell proliferation. Third, crystalline silica particles are taken up by epithelial cells followed by

intracellular generation of free radicals that directly induce genotoxicity. The Working Group considers the first mechanism as the most prominent based on the current experimental data using inhalation or intratracheal instillation in rats, although the other mechanisms cannot be excluded. It is unknown which of these mechanisms occur in humans exposed to crystalline silica dust.' (Silica Dust, Crystalline, in The Form of Quartz or Cristobalite, IARC Volume 100C, p 396 [3])

The predominant commercial categories of silica dust are: sand/gravel, quartz crystals and diatomite (used in filtration and as abrasives). The main route of human exposure is inhalation. Chronic occupational silica exposure produces a fibrotic lung disease called silicosis. Silica dust causes lung cancer.

Silica dust does not directly damage DNA. However, it provokes a strong inflammatory response with macrophage activation and release of cytokines and chemokines. Reactive oxygen species are created by macrophages and through direct chemical interaction with the silicates. Silica particles can be directly phagocytized into lung epithelial cells and generate reactive oxygen species. This leads to DNA damage, including strand breaks. Quartz crystals have been shown to chemically deplete antioxidants in lung tissue. This process is summarized in figure 4.1 of IARC Volume 100C, p 395.

Updated Information

Crystalline silica induces apoptosis in rat macrophages in vitro [130]. An increase in '*hprt*' mutation frequency was detected in alveolar epithelial cells obtained from rats exposed to alpha-quartz [131]. Quartz-treated, transformed and tumorigenic cells exhibited an increase in the expression of transforming growth factor (TGF)-beta1/beta2 mRNA transcripts [132].

Wood dust (Volume 62)

'Potential mechanisms responsible for the carcinogenicity of wood dust include tissue injury induced by the deposition of wood dust particles in the sinonasal region, impaired ciliary clearance, direct genotoxicity and indirect genotoxicity secondary to chronic inflammation. Wood or leather dusts may also act as carrier for other genotoxic agents (e.g. chromate).' (Wood Dust, IARC Volume 100C, p 459 [3])

Dust from wood products is a complex substance that varies considerably depending on the species of tree. Most human exposure arises from woodworking activities in construction, furniture building, etc. Wood dust causes sinonasal cancer (adenocarcinomas) and may cause nasopharyngeal cancer. Evidence suggests that exposure to dust from hardwood tress is associated with a higher risk.

The mechanism of carcinogenesis for wood dust is unknown. The mechanism with greatest support relates to a combination of impaired mucociliary clearance of wood particles from the nose and sinus area leading to mechanical irritation, chronic inflammation and increased cellular proliferation. Direct experimental evidence to support this model is lacking. Extracts from some species of wood (oak and beech) have shown mutagenicity in bacterial and rat hepatocyte systems.

Wood dust is also known to act as a carrier for other carcinogens. There is strong evidence that oak and beech wood contain chromium compounds.

Updated Information

Wood dust exposure induces cytotoxicity and production of reactive oxygen species (ROS) in both animal and human cell lines activate apoptotic caspase-3 enzyme *in vitro* [133,134]. Solvent extracts of natural woods induced chromosome aberrations in respiratory cells in culture [135]. Wood dust exposure modulates macrophage-derived cytokines and chemokines [136].

VOLUME 100D: RADIATION

The mechanisms underlying carcinogenesis from radiation relate to the form of radiation emitted by the agent rather to the specific agent. IARC Volume 100D discusses radiation in separate chapters based on radiation type. The chapter on X- and γ-radiation summarizes mechanistic information related to all types of ionizing radiation. We will follow that general structure. The next section will summarize carcinogenic mechanisms for ionizing radiation; the mechanisms apply to all agents producing ionizing radiation. The agent-specific sections will discusses issues of relevance only to that agent.

General Mechanisms Related to Ionizing Radiation (Group 1 carcinogens listed later) (Volume 75)

'All types of ionizing radiation, including neutron radiation, transfer their energy to biological material in clusters of ionization and excitation events, primarily through a free-electron-mediated mechanism. In cells, energy deposition from all types of ionizing radiation results in a wide variety of molecular damage; in DNA, this includes base damage and single- and double-strand breaks, some of which may be clustered and form complex lesions. Subsequent processing of these lesions may lead to chromosomal aberrations and mutations. Much evidence points to damage to DNA being of primary importance in the biological outcome of exposure to ionizing radiation.....Genome-wide sequencing of tumours has shown wide heterogeneity in constituent mutations, indicating there may be multiple pathways to tumour formation.....There is emerging consensus that epigenetic factors are important in tumorigenic processes. Notably, radiation induces effects such as genomic instability and bystander effects, which are epigenetic in origin. Also important are the interactions at the tissue level between radiation-damaged cells and normal cells.' (reformatted from: Section on X- and γ-radiation, IARC Volume 100D, p209-210)

Higher energy radiation is capable of ionizing molecules. Several types of ionizing radiation are recognized: X-rays, γ-rays, neutron radiation, α-particles and β-particles. The source, energy levels and depth of penetration vary across different types. However, the mechanisms of interaction with tissue, and the carcinogenic mechanisms, are essentially the same.

Everyone is exposed to background radiation from soil, building materials, cosmic rays and radon gas. External radiation exposure accounts for about 40% of total radiation dose with about 50% of that being related to medical procedures. Populations who were exposed to intense ionizing radiation levels from nuclear bomb explosions contribute substantially to knowledge of cancer risk and understanding of carcinogenesis. However, such exposures make only a minor contribution to average exposure. Internal radiation exposure can occur from radionuclides (e.g. radon or iodine-131) deposited into the body through absorption of products from sources such as: natural decay of building materials, by-products of nuclear explosion testing, radiation release events (e.g. Chernobyl), or radiotherapy/diagnostic procedures. Internal exposure occurs mostly from α-particles and β-particles.

Section 4.2.1 (IARC Volume 100D, p 194-197) provides an excellent overview of the general mechanisms associated with radiation-induced carcinogenesis that is summarized in their Figure 4.1 (p198). Two main models have been proposed: the mutational theory (coding changes to DNA) and a non-genetic effect theory (epigenetic factors). Bystander effects have also been proposed.

The mutational theory assumes a carcinogenic model that is purely genetic: ionizing radiation causes damage to DNA. DNA repair processes fail to correct all of the damage, leading to mutations in cells following mitosis. Carcinogenesis results from the accumulation of such damage and clonal expansion.

The non-genetic model is focused on epigenetic factors. These factors affect the dynamic functioning of cells rather than cause coding changes in DNA. A wide range of epigenetic changes has been observed and contribute towards carcinogenesis. Ionizing radiation can cause epigenetic changes.

All three of these mechanisms likely contribute to carcinogenesis from ionizing radiation. The mechanisms will interact in complex ways, with interactions also occurring with remote and nearby normal cells and other host factors.

Ionizing radiation deposits energy to cellular molecules, leading to a wide range of damage. DNA damage can result either from direct ionization of its constituent atoms or indirectly by reactions with free radicals produced by interactions of radiation with water molecules (e.g. the hydroxyl radical). There is strong evidence that ionizing radiation is capable of producing a wide-range of mutations and DNA damage, leading to large-scale gene deletion, gross chromosomal damage and genetic instability. DNA damage can include: base-pair damage, single-strand breaks, double-strand breaks, DNA–protein cross-links, and combinations of these. Observed events include: chromosomal aberrations, gene-sequence and mini-satellite mutations, and apoptosis.

Updated Information

There is strong evidence that ionizing radiation induces mutations in p53 and other loci [137,138,139,140]. Epigenetic effects that are induced by ionizing radiation include alteration in DNA methylation in humans and animals *in vivo [141,142,143]*, and changes in micro RNA expression [142,144]. Ionizing radiation induces telomere shortening and dysfunction in human radiation workers [145]. Similar effects were found in *in vitro* human and animal cell lines [146,147,148,149].

Solar Radiation (Volume 55)

'....it is now known that following exposure to the individual components of UVR [Ultra-violet radiation] .... DNA damage (is) detectable, in particular ... cyclobutane-pyrimidine dimers.... Human cells have DNA-repair pathways that repair DNA photoproducts: the absence of these enzymes, as seen in XP [Xeroderma Pigmentosum] patients, leads to an increase risk of developing squamous cell carcinomas and melanomas lending support to a major role of DNA photoproducts in photocarcinogenesis.....Mutations can be detected in human cells exposed to UVA, UVB and UVC' (Solar and Ultraviolet radiation, IARC Volume 100D, p 89-90)

Sunlight is the main source of ultraviolet radiation exposure for most humans. The carcinogenicity of solar radiation is related to the UV content. The carcinogenic mechanisms are discussed in a subsequent section (Ultraviolet radiation).

Use of UV-emitting Tanning Devices (Volume 55)

'....it is now known that following exposure to the individual components of UVR [Ultra-violet radiation] .... DNA damage (is) detectable, in particular ... cyclobutane-pyrimidine dimers....

Human cells have DNA-repair pathways that repair DNA photoproducts: the absence of these enzymes, as seen in XP [Xeroderma Pigmentosum] patients, leads to an increase risk of developing squamous cell carcinomas and melanomas lending support to a major role of DNA photoproducts in photocarcinogenesis.....Mutations can be detected in human cells exposed to UVA, UVB and UVC' (Solar and Ultraviolet radiation, IARC Volume 100D, p 89-90)

Tanning devices are carcinogenic mainly because they emit ultraviolet radiation. The carcinogenic mechanism is discussed in the next section (Ultraviolet Radiation).

Ultraviolet Radiation (bandwidth 100–400 nm, encompassing UVC, UVB and UVA) (Volume 55)

'....it is now known that following exposure to the individual components of UVR [Ultra-violet radiation] .... DNA damage (is) detectable, in particular ... cyclobutane-pyrimidine dimers.... Human cells have DNA-repair pathways that repair DNA photoproducts: the absence of these enzymes, as seen in XP [Xeroderma Pigmentosum] patients, leads to an increase risk of developing squamous cell carcinomas and melanomas lending support to a major role of DNA photoproducts in photocarcinogenesis.....Mutations can be detected in human cells exposed to UVA, UVB and UVC' (Solar and Ultraviolet radiation, IARC Volume 100D, p 89-90)

Human exposure to Ultraviolet Radiation (UVR) comes primarily through sunlight exposure. Important secondary exposures sources are: tanning beds, UVB phototherapy of psoriasis and welding. UVR causes basal and squamous cell skin carcinoma and melanoma. Causal links to lip carcinoma, and ocular melanoma are strongly suggestive. Evidence is unclear as to the relative carcinogenicity of different wavelengths of UVR (i.e. UVA, UVB and UVC).

High energy UVR (e.g. UVB/C) interacts directly with DNA to produce damage. UVA interacts mainly with endogenous or exogenous photosensitizers, which release reactive oxygen and nitrogen species that lead to DNA damage. A common effect is the production of cyclobutane-pyrimidine dimers between adjacent CC base pairs.

UVR is mutagenic in *in vitro* and *in vivo* testing across a wide range of species. Mutations usually are targeted at pyrimidine–pyrimidine (py–py) units. The most common mutations are tandem CC→TT transitions. DNA damage produced by UVR is repaired through the NER pathway; rare genetic conditions such as Xeroderma Pigmentosum (which impair the NER pathway) are associated with more extensive DNA damage and cancer risk. This DNA damage can lead to genomic instability. A bystander effect[2] has also been reported. UVR exposure has also been reported to cause suppression of certain aspects of the immune system response.

Updated Information

In vitro and *in vivo* testing found that UV induces single strand breaks in human cells [150,151]. In mouse embryonic fibroblasts harbouring a humanized p53 gene, UV induced p53 mutations [152]. UVB induced aberrant DNA methylation and histone modifications in mouse skin and in human squamous cell carcinoma [153]. Histone modifications have been found [154,155]. *In vitro* animal studies have found that UV induces apoptosis [156,157].

[2] A bystander effect is where non-irradiated cells exhibit effects caused by radiation as a result of chemical signals (messengers) received from nearby irradiated cells. Similar effects can be seen in response to non-radiation exposures.

X-radiation and γ-radiation (Volume 75)

The IARC summary was presented in the section on 'General mechanisms related to Ionizing Radiation'. The current material relates to the section: X- and γ-radiation, IARC Volume 100D, p 103.

X-ray and γ-radiation have been established as causal agents for a wide range of cancers, including cancers of the gastrointestinal tract, leukemia, lung, breast, and kidney. The general carcinogenetic mechanisms discussed in the previous section are applicable. X-rays and γ-radiation penetrate matter to a greater depth than do α- and β-particles and have different radiation tracks in tissues. The higher penetrating power increases the range of tissues that they can affect.

Neutron Radiation (Volume 75)

The IARC summary was presented in the section on 'General mechanisms related to Ionizing Radiation'. The current material relates to the section: Neutron radiation, IARC Volume 100D, p 231.

The general carcinogenic mechanisms for neutron particle radiation are similar to those described above. However, neutrons are electrically neutral and interact with the body mainly through interactions with atomic nuclei. This leads to molecular damage being clustered in space, which has been suggested to reduce the effectiveness of DNA repair mechanisms. It should be noted that the IARC monograph found limited evidence that neutron radiation causes cancer in humans; its classification as a Group 1 human carcinogen was based largely on animal studies and the similarity of the biological effects to those associated with X-ray and γ-radiation.

Radon-222 (Volumes 43, 45 & 78)

The IARC summary was presented in the section on 'General mechanisms related to Ionizing Radiation'. The current material relates to the section: Internalized α-particle Emitting Radionuclides, IARC Volume 100D, p 241.

Radon is the main source of α-particle exposure for the general public. It causes lung cancer. Other α-particle emitters include: radium (sarcomas), thorium (liver and leukemia) and plutonium (lung, live and bone).

The general carcinogenic mechanisms are as outlined above. α-particle radiation has a limited ability to penetrate tissue. Internal exposure is necessary for carcinogenesis. α-particles emitted by radionuclides have been shown to cause chromosomal aberrations in circulating lymphocytes and gene mutations in humans *in vivo*.

Radium-224 (Volumes 43, 45 & 78)

The IARC summary was presented in the section on 'General mechanisms related to Ionizing Radiation'. The current material relates to the section: Internalized α-particle Emitting Radionuclides, IARC Volume 100D, p 241.

Radium causes sarcomas.

The general carcinogenic mechanisms are as outlined above. α-particle radiation has a limited ability to penetrate tissue. Internal exposure is necessary for carcinogenesis. α-particles emitted by radionuclides have been shown to cause chromosomal aberrations in circulating lymphocytes and gene mutations in humans *in vivo*.

Radium-226 (Volumes 43, 45 & 78)

The IARC summary was presented in the section on 'General mechanisms related to Ionizing Radiation'. The current material relates to the section: Internalized α-particle Emitting Radionuclides, IARC Volume 100D, p 241.

Radium causes sarcomas.

The general carcinogenic mechanisms are as outlined above. α-particle radiation has a limited ability to penetrate tissue. Internal exposure is necessary for carcinogenesis. α-particles emitted by radionuclides have been shown to cause chromosomal aberrations in circulating lymphocytes and gene mutations in humans *in vivo*.

Radium-228 (Volumes 43, 45 & 78)

The IARC summary was presented in the section on 'General mechanisms related to Ionizing Radiation'. The current material relates to the section: Internalized α-particle Emitting Radionuclides, IARC Volume 100D, p 241.

Radium causes sarcomas.

The general carcinogenic mechanisms are as outlined above. α-particle radiation has a limited ability to penetrate tissue. Internal exposure is necessary for carcinogenesis. α-particles emitted by radionuclides have been shown to cause chromosomal aberrations in circulating lymphocytes and gene mutations in humans *in vivo*.

Thorium-232 (as Thorotrast) (Volumes 43, 45 & 78)

The IARC summary was presented in the section on 'General mechanisms related to Ionizing Radiation'. The current material relates to the section: Internalized α-particle Emitting Radionuclides, IARC Volume 100D, p 241.

Thorium causes live cancer and leukemia.

The general carcinogenic mechanisms are as outlined above. α-particle radiation has a limited ability to penetrate tissue. Internal exposure is necessary for carcinogenesis. α-particles emitted by radionuclides have been shown to cause chromosomal aberrations in circulating lymphocytes and gene mutations in humans *in vivo*.

Plutonium-239 (Volumes 43, 45 & 78)

The IARC summary was presented in the section on 'General mechanisms related to Ionizing Radiation'. The current material relates to the section: Internalized α-particle Emitting Radionuclides, IARC Volume 100D, p 241.

Plutonium causes lung, liver and bone cancer.

The general carcinogenic mechanisms are as outlined above. α-particle radiation has a limited ability to penetrate tissue. Internal exposure is necessary for carcinogenesis. α-particles emitted by radionuclides have been shown to cause chromosomal aberrations in circulating lymphocytes and gene mutations in humans *in vivo*.

Underground Haematite Mining with Exposure to Radon (Volumes 43, 45 & 78)

The IARC summary was presented in the section on 'General mechanisms related to Ionizing Radiation'. The current material relates to the section: Internalized α-particle Emitting Radionuclides, IARC Volume 100D, p 241.

Haematite (iron-ore) mining in underground mines exposes workers to radon from the rock containing the iron ore. Haematite mining causes lung cancer. Radon exposure is believed to be the carcinogenic process.

The general carcinogenic mechanisms are as outlined above. α-particle radiation has a limited ability to penetrate tissue. Internal exposure is necessary for carcinogenesis. α-particles emitted by radionuclides have been shown to cause chromosomal aberrations in circulating lymphocytes and gene mutations in humans *in vivo*.

Internalized radionuclides that emit α-particles (Volumes 43, 45 & 78)

The IARC summary was presented in the section on 'General mechanisms related to Ionizing Radiation'. The current material relates to the section: Internalized α-particle Emitting Radionuclides, IARC Volume 100D, p 241.

This Group 1 classification applies to all α-particle emitting radionuclides not otherwise listed.

The general carcinogenic mechanisms are as outlined above. α-particle radiation has a limited ability to penetrate tissue. Internal exposure is necessary for carcinogenesis. α-particles emitted by radionuclides have been shown to cause chromosomal aberrations in circulating lymphocytes and gene mutations in humans *in vivo*.

Short-lived Radioisotopes of Iodine, Including Iodine-131 (^{131}I) (Volume 78)

The IARC summary was presented in the section on 'General mechanisms related to Ionizing Radiation'. The current material relates to the section: Internalized β-particle Emitting Radionuclides, IARC Volume 100D, p 285.

Iodine-131 is a β-particle emitter that causes thyroid cancer. Children and adolescents are a particular risk.

Based on the similarity of the biological effects to those associated with X-ray and γ-radiation, and the evidence for carcinogenesis for some β-particle emitters, IARC classified all β-particle emitters as Group 1 carcinogens. The general carcinogenic mechanisms are as outlined above in the discussion of the general carcinogenic mechanism for ionizing radiation.

Phorphorus-32 (^{32}P), as Phosphate (Volume 78)

The IARC summary was presented in the section on 'General mechanisms related to Ionizing Radiation'. The current material relates to the section: Internalized β-particle Emitting Radionuclides, IARC Volume 100D, p 285.

Phosphorus-32 is a β-particle emitter that causes leukemia.

Based on the similarity of the biological effects to those associated with X-ray and γ-radiation, and the evidence for carcinogenesis for some β-particle emitters, IARC classified all β-particle emitters as Group 1 carcinogens. The general carcinogenic mechanisms are as outlined above in the discussion of the general carcinogenic mechanism for ionizing radiation.

Mixtures of Fission Products (Volume 78)

The IARC summary was presented in the section on 'General mechanisms related to Ionizing Radiation'. The current material relates to the section: Internalized β-particle Emitting Radionuclides, IARC Volume 100D, p 285.

Fission products include β-particle emitting radionuclides. Based on the similarity of the biological effects to those associated with X-ray and γ-radiation, and the evidence for carcinogenesis for some β-particle emitters, IARC classified all β-particle emitters as Group 1 carcinogens. The general carcinogenic mechanisms are as outlined above in the discussion of the general carcinogenic mechanism for ionizing radiation.

Internalized Radionuclides that Emit β Particles (Volume 78)

The IARC summary was presented in the section on 'General mechanisms related to Ionizing Radiation'. The current material relates to the section: Internalized β-particle Emitting Radionuclides, IARC Volume 100D, p 285.

This Group 1 carcinogen incorporates all β-particle emitting radionuclides, including those already mentioned. Based on the similarity of the biological effects to those associated with X-ray and γ-radiation, and the evidence for carcinogenesis for some β-particle emitters, IARC classified all β-particle emitters as Group 1 carcinogens. The general carcinogenic mechanisms are as outlined above in the discussion of the general carcinogenic mechanism for ionizing radiation.

All Types of Ionizing Radiation (Volume 78)

The IARC summary was presented in the section on 'General mechanisms related to Ionizing Radiation'. The current material relates to the section: Internalized β-particle Emitting Radionuclides, IARC Volume 100D, p 285.

This Group 1 carcinogen presents an encompassing statement concerning all radiation exposure. The general carcinogenic mechanisms are as outlined above in the discussion of the general carcinogenic mechanism for ionizing radiation.

VOLUME 100E: PERSONAL HABITS AND INDOOR COMBUSTIONS

Tobacco Smoking (Volumes 38, 42, 83 & Supplement 7)

'The pathways by which tobacco products cause cancer essentially recapitulate established mechanisms of carcinogenesis by individual compounds [of tobacco smoke]. .... [M]ost carcinogens, either directly, or after metabolism catalyzed by multiple cytochrome P450 enzymes, react with nucleophilic sites in DNA to form ... adducts ... [which], if left unrepaired by cellular DNA repair enzymes ...[cause] permanent mutations..... Multiple studies of mutations in KRAS, p53, and other growth control genes in lung tumours from smokers, some of which report thousands of mutations, are fully consistent with this overall concept.....There are over 70 established carcinogens in cigarette smoke....Multiple DNA adducts are present in the lungs and other tissues of smokers, and sister chromatid exchanges as well as other genetic effects are consistently observed.....There are other processes which contribute to cancer induction by tobacco products....includ[ing] inflammation, tumour promotion, oxidative damage, co-carcinogenesis, and direct activation of cellular growth pathways by constituents of smoke.' (Tobacco smoking, IARC Volume 100E, p 165-6)

Tobacco smoking is a potent cause of cancers at multiple sites, including: lung, oral cavity, naso-, oro- and hypopharynx, nasal cavity and accessory sinuses, larynx, oesophagus, stomach, pancreas, colorectum, liver, kidney (body and pelvis), ureter, urinary bladder, uterine cervix and ovary (mucinous), and myeloid leukaemia. Over 5,300 compounds have been identified in tobacco smoke, including over 70 known carcinogens, on which 16 are Group 1 human carcinogens, including: nitrosamines (e.g. NNK and NNN which are discussed in a later section), polycyclic aromatic hydrocarbons (e.g. benzo(a)pyrene), 1,3-butadiene and cadmium. The carcinogenic mechanisms associated with tobacco smoking reflect those of the constituent carcinogens. It is not feasible to review all of these agents in this section.

A conceptual framework for smoking carcinogenesis is presented in IARC Volume 100E (Figure 4.1, p 133). It largely follows a genetic-damage model. The major pathway involves the binding of carcinogens (or their metabolites) to DNA, forming adducts, leading to mutation in key genes; TP53 and K-RAS are two genes which are commonly found to be mutated. Gene sequencing studies are '*consistent with ... the chronic bombardment of cellular DNA by tobacco smoke carcinogens*' (IARC Volume 100E, p 132). Mutagenicity of urine excreted by smokers, sister chromatid exchanges, micronuclei in buccal cells, and other genetic effects have been consistently observed.

Other carcinogenic effects have been observed, including: direct activation of cellular signalling pathways (see Figure 5, p 146, IARC Volume 100E) and production of oxidative agents and inflammation. In addition, epigenetic mechanisms have been implicated, including: enzymatic methylation of gene promoters, histone modification and RNA-mediated gene silencing.

There is also some evidence that the carcinogenetic mechanisms may be different for some cancer sites. Interaction effects with alcohol intake have also been documented, perhaps due to alcohol enhancing the solubility of tobacco derived carcinogens or due to interactions with metabolic enzymes in the CYP-family.

It has been hypothesized that individuals display differential susceptibility to carcinogens from tobacco smoke risk associated with polymorphic variations in germline DNA. However, the evidence to support this hypothesis, while suggestive, is still equivocal.

Updated Information

Cigarette smoke exposure produces protein adducts both *in vivo* and *in vitro* [158,159]. Components of cigarette smoke interacts with cell receptors and cell signalling pathways to mediate a variety of effects such as, stimulation of DNA synthesis in lung adenocarcinoma [160], and immunosuppression [161]. Some of other established effects of cigarette smoke such as induction of gene expression and genotoxicity may be mediated by aryl hydrocarbon receptor [162,163].

Second-hand Tobacco Smoke (Volume 83)

'Second-hand tobacco smoke' is classified as a separate Group 1 carcinogen. However, IARC Volume 100E does not present a separate discussion of mechanisms of action for second hand tobacco smoke. Rather, this section of the monograph refers to the material presented on the section on 'Tobacco Smoking'. (Second-hand tobacco smoke, IARC Volume 100E, p. 213 & 132)

Second hand tobacco causes lung cancer, and is strongly associated to cancer of larynx and pharynx.

Second hand tobacco smoke is a mixture of exhaled mainstream smoke and side stream smoke (smoke coming from the tip of the cigarette/cigar while no inhalation is taking place). The chemical composition of second-hand smoke is qualitatively similar to mainstream smoke inhaled directly by the smoker. There are quantitative differences in the concentrations of carcinogens. In addition, human exposure to second hand tobacco smoke is affected by dispersion in the air of the environment, leading to lower doses of exposure.

The carcinogenic mechanisms for second-hand smoke are the same as for tobacco smoking and are discussed in that section.

Smokeless Tobacco (Volumes 37, 42, 52, 89 & Supplement 7)

'Th[e]... conceptual model [for tobacco smoking] can be applied to smokeless tobacco products. Smokeless tobacco products have much lower levels of carcinogens and toxicants that result from combustion, so the effects of these agents are not seen to a significant extent. The most prevalent strong carcinogens in smokeless tobacco are: tobacco-specific nitrosamines, other nitrosamines, PAHs, aldehydes, metals and large amounts of some inorganic salts. .... Multiple studies demonstrate that tobacco specific nitrosamines are absorbed and metabolized in smokeless tobacco users. There is evidence for DNA adduct formation in oral tissues of smokeless tobacco users, and sister chromatid exchanges, chromosomal aberrations, and micronuclei – consequences of DNA adduct formation – have been reported. Many studies have demonstrated RAS and TP53 mutations in smokeless tobacco users. Oxidative stress and reactive oxygen species could play a significant role in cancer induction in smokeless tobacco users, particularly at high pH. Chronic local inflammation and irritation induced by smokeless tobacco. (Smokeless tobacco, IARC Volume 100E, p. 265 & 132)

Smokeless tobacco products can be inhaled or applied orally (chewed, sucked, gargled or applied directly to the gums). The chemical composition depends on the method of manufacture used. Smokeless tobacco causes cancer of the oral cavity, oesophagus and pancreas.

Multiple carcinogens have been identified in smokeless tobacco products, including: tobacco-specific nitrosamines, other nitrosamines, PAHs, and aldehydes. The carcinogenic mechanisms for smokeless tobacco will reflect that of these carcinogens. DNA adduct formation has been

documented in oral tissues and other evidence of chromosomal damage (e.g. sister chromatid exchanges and micronuclei). Mutations in RAS and TP53 have been documented. Localized inflammation and oxidative stress have been identified, with production of reactive oxygen species.

4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-Nitrosonornicotine (NNN) (Volumes 17, 37, 68, 89 & Supplement 7)

'NNK and NNN are the most abundant strong carcinogens in smokeless tobacco; their uptake and metabolic activation has been clearly documented in smokeless tobacco users. Combined application of NNN and NNK to the oral mucosa of rats induced oral tumours, consistent with their induction by smokeless tobacco. One of the mechanisms of carcinogenicity is cytochrome-P450-mediated α-hydroxylation, which leads to the formation of DNA and haemoglobin adducts that have been detected in users of tobacco.' (N'-Nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, IARC Volume 100E, p 328)

4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-Nitrosonornicotine (NNN) are present in all forms of tobacco as a by-product of the curing process. There is limited direct evidence of carcinogenicity in humans, largely due to the difficulty in isolating the direct effect of NNK or NNN from that of other carcinogens present in tobacco smoke. There is very strong evidence of carcinogenicity in animals.

NNK forms Pyridyloxobutyl (POB)-DNA adducts at the 7- and O_6-positions of deoxyguanosine dG, the O_2-position of thymidine, and the O_2-position of deoxycytidine. Metabolic activation of NNK by CYP450 enzymes also leads to 7-methyl-dG and O_6-methyl-dG.

Nicotine, NNK, and similar chemicals bind to nicotinic and other cellular receptors, resulting in activation of serine/threonine kinase Akt, protein kinase A, and other changes. NNK increases expression of survivin and can cause decreased apoptosis, increased angiogenesis, and increased cell transformation.

Updated Information

See tobacco smoking section.

Betel Quid with Added Tobacco (Volumes 37, 85 & Supplement 7)

'Betel quid and areca-nut ingredients and extracts exert a variety of genetic and related effects.... Continuous local irritation of buccal epithelial cells....can generate chronic inflammation, oxidative stress and cytokine production. Reactive oxygen species generated during chewing .... can lead to DNA- and genetic damage in exposed oral keratinocytes. Persistent oxidative stress can drive affected cells to uncontrolled proliferation and hyperplastic/dysplastic lesions.' (Betel Quid and Areca Nut, IARC Volume 100E, p 363)

A Betel quid is an assembled biological substance that is placed in the mouth and chewed or sucked and often swallowed. It is made of a folded betel leaf containing various products such as areca nut, tobacco, slaked lime, and catechu. Chewing betel quid, with or without tobacco products, causes cancer of the oral cavity, pharynx and oesophagus.

Betel quids that contain tobacco products would be subject to the same carcinogenic processes as described for smokeless tobacco. In addition, the other components of a betel quid could be carcinogenic.

Other components of a betel quid (arecoline, betel leaf, slaked lime, and catechu) have been studied for carcinogenesis. Secondary and tertiary amines in betel quids are converted to nitrosamines in the saliva and stomach. There is no evidence that the non-tobacco components of betel quids cause direct DNA damage. However, irritation of oral tissues from the quid, combined with the generation of reactive oxygen species, can produce indirect DNA damage. Slaked lime produces a high pH environment that exacerbates these effects. Human testing has found elevated levels of sister-chromatid exchanges and micronuclei formation. P53 is downregulated and the function of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) is altered.

Betel Quid without Added Tobacco (Volumes 37, 85 & Supplement 7)

'Betel quid and areca-nut ingredients and extracts exert a variety of genetic and related effects.... Continuous local irritation of buccal epithelial cells....can generate chronic inflammation, oxidative stress and cytokine production. Reactive oxygen species generated during chewing .... can lead to DNA- and genetic damage in exposed oral keratinocytes. Persistent oxidative stress can drive affected cells to uncontrolled proliferation and hyperplastic/dysplastic lesions.' (Betel Quid and Areca Nut, IARC Volume 100E, p 363)

Betel quids that do not contain tobacco cause cancer of the oral cavity and oesophagus.

The carcinogenic mechanisms are summarized in a previous section ('Betel Quid with Added Tobacco'.

Areca nut (Volumes 37, 85 & Supplement 7)

'Betel quid and areca-nut ingredients and extracts exert a variety of genetic and related effects.... Continuous local irritation of buccal epithelial cells....can generate chronic inflammation, oxidative stress and cytokine production. Reactive oxygen species generated during chewing .... can lead to DNA- and genetic damage in exposed oral keratinocytes. Persistent oxidative stress can drive affected cells to uncontrolled proliferation and hyperplastic/dysplastic lesions.' (Betel Quid and Areca Nut, IARC Volume 100E, p 363)

Areca nut, the fruit of a palm tree, is the major constituent of a betel quid. It contains several alkaloids and tannins. Arecoline is the most common alkaloid in areca nut. It also contains a variety of amines that are nitrosated in saliva during quid chewing. These all contribute towards carcinogenicity.

Aqueous extracts of areca nut produces a variety of cellular effects including: DNA strand breaks, sister chromatid exchanges, micronucleus formation, gene mutations, chromosomal aberrations and increased cellular proliferation. These were found in both *in vitro* and *in vivo* animal testing. Similar effects were observed from areca nut alkaloids. Arecoline inhibited TP53 expression in human epithelial cells. Irritation of oral tissues from the quid, combined with the generation of reactive oxygen species, can produce indirect DNA damage.

Updated Information

Betel quid and areca nut can increase the expression of calcium binding proteins, believed to be involved in oral submucous fibrosis [164]. They can up-regulate cyclooxygenase inflammatory signalling in the oral cavity [165]. Areca nut induces the TGF-β pathway during the progression of oral submucous fibrosis [166].

Alcohol Consumption (Volumes 44 & 51)

The IARC Volume 100E mechanistic summary is too complex to quote. It describes potential carcinogenetic mechanisms for ethanol and acetaldehyde. The information is summarized in the following material. (Consumption of Alcoholic Beverages, IARC Volume 100E, p470-1)

The term 'Alcohol beverages' refers to a wide group of beverages that are produced by fermentation. Alcohol causes several cancers: oral cavity, pharynx, larynx, oesophagus, colorectum, liver (hepatocellular carcinoma) and female breast.

While alcohol beverages contain many ingredients, not all of which are present in all types of alcohol, all forms of alcohol share carcinogenic potential. Hence, the main focus for cancer risk has been on ethanol (the primary non-water constituent of alcohols) and acetaldehyde (the initial product in the metabolism of ethanol in the human body). In addition to potential direct carcinogenicity, alcohol can act as an agent to facilitate the transport of other carcinogens into the body. That mechanism will not be considered further.

Ethanol intake produces increased reactive oxygen species and oxidative stress, which damage DNA and affect its repair. Ethanol causes hepatocellular injury, fibrotic changes and cirrhosis. Reduced folate levels are associated with alcohol intake but may reflect general life-style factors rather than a biological mechanism. In animals *in vivo*, ethanol induced DNA adducts, DNA strand breaks, and sister chromatid exchanges and dominant lethal mutations.

Acetaldehyde causes DNA-adducts, DNA–protein crosslinks, DNA strand breaks, sister chromatid exchanges, chromosomal aberrations, and micronuclei in eukaryotic cells in vitro. There is evidence for gene-environment interaction in cancer risk with regard to the ALDH2 and ADH1B genes.

Alcoholics have been found to have significantly higher levels of chromosomal aberrations and cells with micronuclei than either non-drinking controls or abstinent alcoholics.

Updated Information

Ethanol induces epigenetic changes [167,168,169,170,171] and histone modifications [167,172,173,174]. There is evidence of increased expression of miRNAs in humans [175,176]. Some studies in humans showed genotype susceptibility to alcohol-induced oesophageal cancer [177,178].

Ethanol in Alcoholic Beverages (Volumes 44 & 51)

The IARC Volume 100E mechanistic summary (p. 470-1) is too complex to quote. It describes potential carcinogenetic mechanisms for ethanol and acetaldehyde. The information is summarized in the following material. (Consumption of Alcoholic Beverages, IARC Volume 100E, p470-1)

Ethanol intake produces increased reactive oxygen species and oxidative stress, which damage the DNA and affect its repair. Ethanol causes hepatocellular injury, fibrotic changes and cirrhosis. Reduced folate levels are associated with alcohol intake but may reflect general life-style factors rather than a biological mechanism. In animals *in vivo*, ethanol induced DNA adducts, DNA strand breaks, and sister chromatid exchanges and dominant lethal mutations.

Alcoholics have been found to have significantly higher levels of chromosomal aberrations and cells with micronuclei than either non-drinking controls or abstinent alcoholics.

Updated Information

See alcohol beverage section for update information.

Acetaldehyde Associated with the Consumption of Alcoholic Beverages (Volumes 44 & 51)

The IARC Volume 100E mechanistic summary (p. 470-1) is too complex to quote. It describes potential carcinogenetic mechanisms for ethanol and acetaldehyde. The information is summarized in the following material. (Consumption of Alcoholic Beverages, IARC Volume 100E, p470-1)

Acetaldehyde causes DNA-adducts, DNA–protein crosslinks, DNA strand breaks, sister chromatid exchanges, chromosomal aberrations, and micronuclei in eukaryotic cells in vitro. There is evidence for gene-environment interaction in cancer risk with regard to the ALDH2 and ADH1B genes.

Alcoholics have been found to have significantly higher levels of chromosomal aberrations and cells with micronuclei than either non-drinking controls or abstinent alcoholics.

Chinese-style Salted Fish (Volume 56)

'Possible mechanisms for the association of consumption of Cantonese-style salted fish with risk of NPC [naso-pharyngeal carcinoma] are the formation endogenously of N-nitroso compounds in the human body and/or their formation due to the processing of the fish — i.e. a reaction between secondary and tertiary amines in the fish and nitrate/nitrite in the crude salt used — and activation of the oncogenic Epstein-Barr virus. These two mechanisms are not mutually exclusive.' (Chinese-style salted fish, IARC Volume 100E, p 510)

Chinese-style salted fish has been established as a cause of nasopharyngeal carcinoma.

The carcinogenic mechanisms are not well established. DMSO extracts of salted fish were mutagenic in *S. typhimurium* TA 100 and TA 98 in one small study in the presence of rat liver metabolic activation system. However, n-hexane and ethyl acetate extracts were not mutagenic in these systems, even in the presence of metabolic activation systems. Chinese-style salted fish contains high levels of amines that are converted to nitrosamine compounds either during the processing of the fish or when it is consumed. These nitrosamine compounds are directly genotoxic. There is also evidence that Chinese-style salted fish can re-activate Epstein Barr virus, which is known to cause nasopharyngeal carcinoma.

Updated Information

Taj et al [179] found evidence of mutations in *S. typhimurium* TA100 and TA98 Ames testing with DMSO extracted samples (metabolic activation required) and XAD extraction. *In-vivo* testing in rats found evidence of cytogenic damage and DNA strand breaks, especially in hepatocytes. Fong et al [180], in addition to the DMSO testing mentioned in the IARC monograph, reported that the urine of rats fed salted fish were mutagenic in Ames testing.

Indoor Emissions from Household Combustion of Coal (Volume 95)

'Chemical analyses and bioassay-directed fractionation of smoky coal emissions have identified PAHs as an important chemical class that accounts for much of their mutagenicity and carcinogenicity. The epidemiological link between exposure to smoky coal emissions and an increased risk for lung cancer is strengthened mechanistically by the fact that the mutation spectra

of the P53 tumour-suppressor gene and the KRAS oncogene in the lung tumours from non-smokers exposed to smoky coal emissions reflect an exposure to PAHs and differs from the mutation spectra found in these genes in lung tumours from cigarette smokers. Thus, the mutation spectra in lung tumours from non-smokers whose cancers are linked to smoky coal emissions reflect the primary DNA damage induced by the most prominent class of mutagens/carcinogens in these emissions'. Indoor emissions from household combustion of coal, (IARC Volume 100E, p532)

Household combustion of coal causes lung cancer.

Combustion of coal in household appliances such cooking and heating stoves leads to incomplete combustion that produces by-products including polycyclic aromatic hydrocarbons (PAHs) and aldehydes. In addition, combustion releases contaminants such as silicates, sulphur, and mercury.

PAHs are the main carcinogenic agents from coal emissions. PAHs are metabolized rapidly to more soluble metabolites and reactive species (e.g. phenols, dihydrodiols, epoxides, quinones and tetrols). These compounds interact with DNA to produce DNA adducts. Metabolism to PAH-epoxides is a major component of the carcinogenic mechanism. Other potential mechanisms include production of reactive oxygen species, interruption of gap-junctional intercellular communication, cell-cycle dysregulation, induction of apoptosis and immunosuppression. Some of these effects may be mediated by activation of the aryl-hydrocarbon receptor.

VOLUME 100F: OCCUPATIONAL CARCINOGENS

4-Aminobiphenyl (Volumes 1, 42, 99 & Supplement 7)

'There is strong mechanistic evidence indicating that the carcinogenicity of 4-aminobiphenyl in humans operates by a genotoxic mechanism of action that involves metabolic activation, formation of DNA adducts, and induction of mutagenic and clastogenic effects. Metabolic activation to DNA-reactive intermediates occurs by multiple pathways including N-oxidation in the liver, O-acetylation in the bladder, and peroxidative activation in the mammary gland and other organs.' (4-aminobiphenyl, IARC Volume 100F, p 50)

4-Aminobiphenyl was used mainly in industrial settings as a rubber antioxidant, as a dye intermediate, and in the detection of sulphates. Its use in industry was phased out in the mid-1950's. Non-industrial exposure occurs primarily through smoking. Industrial exposure to 4-aminobiphenyl causes bladder cancer.

4-Aminobiphenyl is a member of the aromatic amine family. It shares carcinogenic mechanisms with other members of the family. A recent review of carcinogenic mechanisms for aromatic amines is found in Volume 99 of the IARC monograph series. The genotoxic effects of aromatic amines (including 4-aminobiphenyl) are '*well established on the basis of mutagenicity and clastogenicity observed in numerous in vitro and in vivo assays that show the capability of these compounds to form DNA adducts after metabolic activation to electrophilic intermediates*' (IARC Volume 100F, p 48).

4-Aminobiphenyl is activated through N-hydroxylation in the liver. The resulting metabolite is highly electrophilic and forms DNA adducts. The N-hydroxy metabolite is glucuronidated and excreted through the kidney. However, the acidic environment in the bladder lumen re-creates the N-hydroxy metabolite. NAT1-mediated O-acetylation occurs which lead to the production of highly reactive aryl nitronium ions and DNA adducts.

Mutations were induced when 4-aminobiphenyl was tested on *S. typhimurium* in the presence of S-9-mediated metabolic activation. 4-aminobiphenyl induced mutations at the HPRT locus and chromosomal instability in human bladder epithelial cells. 4-Aminobiphenyl induced mutations have been found in h-RAS (mice) and TP53 (human bladder cells).

Benzidine (Volumes 1, 29, 99 & Supplement 7)

'There is strong mechanistic evidence indicating that the carcinogenicity of benzidine in humans operates by a genotoxic mechanism of action that involves metabolic activation, formation of DNA adducts, and induction of mutagenic and clastogenic effects. Metabolic activation to DNA-reactive intermediates occurs by multiple pathways including N-oxidation in the liver, O-acetylation in the bladder, and peroxidative activation in the mammary gland and other organs.' (Benzidine, IARC Volume 100F, p61)

Benzidine is an aromatic amine with a long history of use in the production of dyes, particularly azo dyes used with wool, cotton and leather. Industrial production has been phased out since the 1960's, with bans on its use in many countries. Most exposure is from occupational contact. The general public may have low exposure through contact with consumer goods that still use azo-based dyes. Benzidine causes bladder cancer.

The general metabolism and carcinogenic mechanisms are similar to those discussed on the section on 4-Aminobiphenyl.

Workers exposed to benzidine or benzidine-based dyes have significant increases in chromosomal aberrations in peripheral lymphocytes and P53 mutations. Rats exposed to benzidine had TP53 mutations the bladder, liver, and lung cells with an increased level of micronuclei in bone-marrow cells and strand-breaks in the liver.

Updated Information

Benzidine induces apoptosis, cell proliferation and differentiation in animals [181,182].

Dyes Metabolized to Benzidine (Volume 99 & Supplement 7)

'There is strong mechanistic evidence indicating that benzidine-based dyes are converted by azoreduction to benzidine in humans and in experimental animals and, consequently, produce DNA adducts and genotoxic effects similar to those of benzidine.' (Dyes metabolized to Benzidine, IARC Volume 100F, p 71)

This section discussed three dyes: Direct Black 38, Direct Blue 6 and Direct Brown 95. There is a lack of direct epidemiological evidence that these dyes cause cancer in humans. However, animal studies show that they are carcinogenic and there is strong evidence that these dyes are metabolized to benzidine, a known carcinogen in humans.

The carcinogenic mechanisms are the same as for the active metabolite benzidine.

4,4′-Methylene bis(2-chlorobenzenamine) (Volumes 4, 42, 57, 99 & Supplement 7)

'There is strong mechanistic evidence indicating that the carcinogenicity of 4,4′-methylenebis(2-chlorobenzenamine) involves a genotoxic mechanism of action that includes metabolic activation, formation of DNA adducts, and induction of mutagenic and clastogenic effects in humans. Metabolic activation to DNA-reactive intermediates occurs by multiple pathways including N-oxidation in the liver, O-acetylation in the bladder, and peroxidative activation in the mammary gland and other organs.....The genotoxicity of 4,4′-methylenebis(2-chlorobenzenamine) is well documented.... It has also been shown to cause the formation of sister chromatid exchange and micronuclei in urothelial cells and lymphocytes of exposed workers.' (4,4'-Methylene bis(2-chlorobenzenamine), IARC Volume 100F, p 80)

4,4′-Methylenebis(2-chlorobenzenamine) (MOCA) is used as a curing agent in the production of some types of polyurethane. Industrial exposure is the most common route of exposure although environmental contamination has created some exposure risk for the general public. There is a lack of direct epidemiological evidence that MOCA causes cancer in humans. However, it is a multi-organ carcinogen in experimental animals.

MOCA is a member of aromatic amine family and shares carcinogenic mechanisms with other members (see section on 4-aminobiphenyl). According to NIOSH, this substance is also known as 4,4′-Methylene bis(2-chloroaniline), the name which was used in previous IARC reviews. Acronyms include: MOCA or MBOCA.

N-oxidation of MOCA leads to the production of DNA adducts and mutagenesis. Elevated levels of micronuclei have been noted in exfoliated bladder epithelial cells and in peripheral lymphocytes of workers exposed to MOCA. Exposure causes multiple changes in animal models, including:

unscheduled DNA synthesis, cellular transformation, sister chromatid exchanges, prophage induction in E. coli, and caused aneuploidy in *S. cerevisiae*.

2-Naphthylamine (Volumes 4, 99 & Supplement 7)

'There is strong mechanistic evidence indicating that the carcinogenicity of 2-naphthylamine operates by a genotoxic mechanism of action that involves metabolic activation, formation of DNA adducts, and induction of mutagenic and clastogenic effects. Metabolic activation to DNA-reactive intermediates occurs by multiple pathways including N-oxidation in the liver, O-acetylation in the bladder, and peroxidative activation in the mammary gland and other organs.' (2-Naphthylamine, IARC Volume 100F, p 90)

2-Naphthylamine is used in industrial processes for the manufacture of dyes and rubber. However, its use was been banned in EU in 1998. In the USA, it is a regulated carcinogen. It is also produced from burning tobacco (smoking), cooking oils and similar substances. It causes bladder cancer.

2-Naphthylamine is a member of aromatic amine family and shares carcinogenic mechanisms with other members (see section on 4-aminobiphenyl). 2-Naphthylamine was mutagenic in *S. typhimurium* strains TA98 and TA100 in the presence of bovine bladder cells. 2-Naphthylamine was mutagenic in Chinese hamster ovary cells in the presence or absence of an exogenous activating system.

Ortho-Toluidine (Volume 16, 27, 68, 77, 99 & Supplement 7)

'There is moderate mechanistic evidence indicating that the carcinogenicity of ortho-toluidine involves metabolic activation, formation of DNA adducts, and induction of DNA-damaging effects.' (Ortho-Toluidine, IARC Volume 100F, p 98)

ortho-Toluidine is used in a variety of industrial processes including the production of herbicides and dyes. Exposure is highest in occupational settings with no-occupational exposure mainly through tobacco smoking and hair dyes. *ortho*-Toluidine has consistently been associated with an elevated risk of bladder cancer in humans.

ortho-Toluidine is a member of aromatic amine family and shares carcinogenic mechanisms with other members (see section on 4-aminobiphenyl). The metabolism of *ortho*-Toluidine is still under research. It has been suggested that carcinogenesis may involve peroxidative activation of the chemical, catalyzed by prostaglandin H synthase in the epithelium of the urinary bladder.

'*The N-oxidized metabolite of ortho-toluidine, N-hydroxy-ortho-toluidine, was mutagenic in S. typhimurium strain TA100. Other reported effects of ortho-toluidine include the induction of sister chromatid exchange, aneuploidy, unscheduled DNA synthesis, DNA strand breaks, and cell transformation in vitro, and the induction of micronuclei in peripheral blood of rats treated in vivo.*' (IARC Volume 100F, p 98).

Auramine Production (Volume 1, 42, 99 & Supplement 7)

'There are insufficient mechanistic data relevant to the carcinogenicity of auramine in humans. Auramine induces DNA strand-breaks in experimental animals.' (Auramine and Auramine production, IARC Volume 100F, p 104)

Auramine and its salts are used largely in the production of dyes, which have major application of dyeing paper. Use has been banned in many countries. Auramine causes bladder cancer.

Auramine is a member of aromatic amine family and shares carcinogenic mechanisms with other members (see section on 4-aminobiphenyl). The metabolism of auramine has not been studied. However, commercial preparations of auramine were mutagenic in several strains of *S. typhimurium*, when tested with metabolic activation systems. *In vitro* studies have demonstrated: DNA strand breaks, unscheduled DNA synthesis, micronucleus formation and induction of deletions and aneuploidy in *S. cerevisiae.* DNA strand breaks had been confirmed in *in vivo* studies with rats.

Magenta Production (Volumes 4, 42, 57, 99 & Supplement 7)

'There are insufficient mechanistic data relevant to the carcinogenicity of magenta in humans or experimental animals.' (Magenta and magenta production, IARC Volume 100F, p 109)

Magenta refers to a group of four dyes: Basic Red 09, Rosanilin, Magenta II and New Fuchsin. Their chemical structures are similar. Workers involved with manufacturing the dyes are the main group who are exposed. Magenta causes bladder cancer.

The Magenta dyes are members of the aromatic amine family and share carcinogenic mechanisms with other members (see section on 4-aminobiphenyl).

The metabolism of magenta dyes has not been directly studied and no studies on the specific carcinogenic mechanisms of magenta have been published. Magenta was mutagenic in *S. typhimurium* strains TA98, TA100, and TA1535 when tested in the presence of metabolic activation.

Benzo[a]pyrene (Volumes 3,32,68,92 & Supplement 7)

'Benzo[a]pyrene is metabolically activated to a series of reactive intermediates by CYP450 and related enzymes under control of the arylhydrocarbon receptor.....Benzo[a]pyrene is pleotropic and has the ability to affect many cell- and organ-based systems. Therefore, there are probably many modes of carcinogenic action operating to different extents in vivo. These include mechanisms that involve AhR, oxidative stress, immunotoxicity and epigenetic events.....The genotoxic mechanism of action of benzo[a]pyrene involves metabolism to highly reactive species that form covalent adducts to DNA. These anti-benzo[a]pyrene-7,8-diol- 9,10-oxide-DNA adducts induce mutations in the K-RAS oncogene and the TP53 tumour suppressor gene in human lung tumours, and in corresponding genes in mouse-lung tumours. Exposure[s].... induce other genotoxic effects, including sister chromatid exchange, micronuclei, DNA damage and 8-oxodeoxyguanosine....' (Benzo[a]pyrene, IARC Volume 100F, p 137-8)

Benzo[a]pyrene is a member of the polycyclic aromatic hydrocarbon (PAH) family. It is a widespread environmental contaminant, produced through the incomplete combustion of organic material. High levels are found in tobacco smoke. The IARC 100F monograph reported no epidemiological data on cancer risk in humans specifically from benzo[a]pyrene. However, benzo[a]pyrene containing mixtures have been linked to human tumours of the lung, bladder, oesophagus and lymphatic system. There is strong evidence of multi-site carcinogenesis in animals.

A variety of potential carcinogenic mechanisms have been identified for benzo[a]pyrene. Most involve the initial metabolic transformation of benzo[a]pyrene into reactive intermediaries or ultimate carcinogens such as benzo(a)pyrene-7,8-diol-9,10-epoxides. Key mechanisms include: creation of stable and depurinating DNA adducts, repetitive redox cycling which generates

reactive oxygen species, interaction with the Aryl hydrocarbon receptor (AhR), immunosuppression and epigenetic changes. Binding to the AhR cause perturbations in cell signalling.

Benzo[a]pyrene causes single strand DNA breaks and mutations in p53 in vitro. Benzo[a]pyrene and/or its metabolites have been shown to increase cell proliferation in several human cell lines.

Updated Information

Benzo[a]pyrene has been found to induce hypermethylation in humans and animals [183,184,185,186,187] . It increases the expression of miRNAs in human cells *in vitro* [147,188].

Coal Gasification (Volume 34, 92 & Supplement 7)

'There is strong evidence from experimental studies for a genotoxic mode of action for coal gasification samples. Although there are no human studies, it is highly likely that genotoxicity is the mechanism relevant to the carcinogenic hazards from exposures to emissions of coal gasification.' (Coal gasification, IARC Volume 100F, p 150)

Coal gasification is a process of converting coal into a gaseous mixture that can be burned with lower environmental impact. Coal is reacted with oxygen, steam and carbon dioxide. Several types of gasifiers are used which can expose workers to multiple carcinogenic substances, including: PAHs, arsenic, asbestos, silica, cadmium, nickel and hydrocarbons. Coal gasification causes lung cancer.

Carcinogenic mechanisms reflect those of the released substances. The PAH and alkylated by-product fractions were mutagenic in *S. typhimurium* in the presence of an exogenous metabolic activation system. Mice fed a diet containing coal tar from a gas plant residue showed a complex pattern of aromatic adducts in multiple tissues. Topical application also produced DNA adducts. Several of the PAHs found in ambient air in gas works are mutagenic (i.e. benz[a]anthracene, benzo[a]pyrene, benzo[ghi]perylene) and carcinogenic (i.e. benz[a]anthracene, benzo[a]pyrene). Despite the absence of human studies, a genotoxic mechanism of carcinogenesis has been established.

Occupational Exposures During Coal-tar Distillation (Volume 92)

'Studies in experimental systems and in tissues of humans provide strong evidence for a genotoxic mechanism underlying the effects of occupational exposures during coal-tar distillation in humans. The detection of anti-benzo[a] pyrene-7,8-diol-9,10-epoxide-DNA adducts in the peripheral blood lymphocytes of exposed humans suggests the participation of benzo[a] pyrene in the genotoxic mechanism of this exposure in humans.' (Occupational exposures during coal-tar distillation, IARC Volume 100F, p 158)

This section discusses occupational exposure during distillation of coal-tar; the next section discusses the carcinogenicity of coal-tar pitch as a product. Coal-tar pitch is produced by the distillation of coal-tar which is itself obtained from the condensation of gasses produced through the destructive distillation of coal.

Coal-tar is a complex mixture of condensed-ring aromatic hydrocarbons. Workers involved with coal-tar distillation are exposed to a wide range of PAHs. Coal-tar is sometimes used for the treatment of psoriasis. The coal-tar distillation process has been causally linked to skin cancer with a particular link to scrotal cancer in men.

Coal-tar pitch and roofing-tar emissions were found to be mutagenic in *S. typhimurium* (in the presence of an exogenous metabolic activation system) and in two mammalian cell systems (in the presence and absence of an exogenous metabolic activation system). They induced sister chromatid exchange in Chinese hamster ovary cells and enhanced viral transformation in Syrian hamster embryo cells (both in the absence and presence of an exogenous metabolic activation system). Coal tar applied topically to the skin of male mice produced a complex pattern of DNA adducts and strongly increased the mutation frequency in lambda lacZ transgenic mice (MutaMouse®). DNA strand-breaks were found in exposed mice.

The urine from patients undergoing coal-tar treatments was mutagenic in bacteria. Peripheral blood lymphocytes of workers occupationally exposed to coal tars had increased chromosomal damage.

Coal-tar Pitch (Volumes 35, 92 & Supplement 7)

'There is strong evidence from experimental data that coal-tar pitch has a genotoxic mechanism of action. There is moderate evidence in humans for a genotoxic mechanism underlying the effects of exposures during roofing and paving with coal-tar pitch, based on one study.' (Coal-tar pitch, IARC Volume 100F, p 165)

As noted in the previous section, coal-tar pitch in manufactured through the distillation of coal-tar. It is used in the roofing and paving industries. Exposure during roofing work occurs both when the old roofing material is removed and the new is applied. Coal-tar application for paving was phased out in most of Europe between 1963 and 1995. Coal-tar pitch causes lung cancer.

Carcinogenic mechanisms copy those of the constituent agents. Coal-tar pitch and roofing-tar emissions were mutagenic in bacteria in the presence of an exogenous metabolic activation system, and in mammalian cells with and without metabolic activation. These agents also induced sister chromatid exchange in Chinese hamster ovary cells. Coal tar pitches contained several polycyclic aromatic hydrocarbons that are genotoxic and carcinogenic in experimental studies. In human studies, increasing the dose of exposure was associated with higher levels of DNA strand-breaks.

Coke Production (Volume 34, 92 & Supplement 7)

'Overall, these data strongly indicate a mutagenic/ genotoxic mode of action for occupational exposures during coke production, based on experimental and human studies. ... There is ample mechanistic support for the respiratory carcinogenic effects of occupational exposures during coke production in humans, in part through analysis of exposure to benzo[a]pyrene. This is based on direct measurement of anti-benzo[a]pyrene- 7,8-diol-9,10-oxide-DNA adducts in peripheral blood lymphocytes (surrogate tissue) and on the identification of genotoxic effects consistent with those induced by anti-benzo[a]pyrene-7,8-diol- 9,10-oxide or benzo[a]pyrene. It is also consistent with the known carcinogenic activity of this epoxide in lung tissues in experimental animals. Moreover, the influence of GST polymorphisms on levels of anti-benzo[a]pyrene-7,8-diol-9,10- oxide-DNA adducts is suggestive of the presence of reactive electrophilic intermediates, such as anti-benzo[a]pyrene-7,8-diol-9,10-oxide. Since coke-oven emissions are complex mixtures, these exposures could have more than one underlying mechanism of action. The fact that chronic exposure to PAH in Polish non-smoking coke oven workers induced both gene-specific (e.g. in the TP53 gene) and global methylation changes in peripheral blood lymphocytes, suggests an epigenetic mechanism.' (Coke production, IARC Volume 100F, p175)

Coke is produced from coal and is used as a fuel and reducing agent in iron foundry industrial processes. Production creates exposures to coal dust, various gaseous by-products (e.g. coke-oven gas) and various minerals and other contaminants. Coke production causes lung cancer.

Coke-oven emissions are complex mixtures and there may be more than one underlying carcinogenic mechanism. Coke-oven emissions are mutagenic in bacteria and in mammalian cells, inducing DNA damage, sister chromatid exchanges and morphological cell transformation. Exposure to coke-oven emissions produced benzo(a)pyrene diolepoxide (BPDE) DNA adducts in mice. In humans, peripheral blood lymphocytes of coke-oven workers had increased frequencies of sister chromatid exchange. The urine of coke-oven workers was mutagenic in *S. typhimurium* in the presence of an exogenous metabolic system. There is some evidence that an epigenetic mechanism may be involved in carcinogenicity.

PAHs are one component of coke-oven emissions. As discussed elsewhere, many PAHs are genotoxic in *in-vitro* and *in-vivo* bioassay systems.

Untreated or Mildly Treated Mineral Oils (Volume 3, 33, 42 & Supplement 7)

'There is weak evidence on the mechanism underlying the effects in humans of exposures to mineral oils. This evidence is based on genotoxic (mutagenic) activity of mineral oils in bacteria and a single cytogenetic study of glassworkers exposed to aerosols of mineral oils.' (Mineral oils, untreated or mildly treated, IARC Volume 100F, p 193)

Mineral oils are produced from crude petroleum oils using a wide range of complex chemical processes. The final products are a heterogeneous group of complex chemical mixtures. PAHs are common components, unless the mineral oil is highly refined. Untreated mineral oils (i.e. those which are not highly refined) cause skin cancer. Highly refined mineral oils have not been linked to human cancer.

The carcinogenic mechanism for mineral oils has not been extensively studied. The presence of PAHs in untreated mineral oil may provide the basis for a genotoxic carcinogenic mechanism. Chromosomal damage, including chromatid breaks, chromosome breaks, and chromosome exchanges were observed in a group of 31 male glassmakers (smokers and non-smokers). In laboratory studies, the mutagenic activities of the mineral-oil samples were significantly correlated with the amount of 3-7-ring polycyclic aromatic compounds for a subgroup of oil samples.

Shale Oils (Volume 35 & Supplement 7)

'Shale oils are genotoxic in experimental systems. There are only few data to determine an underlying mechanism for the carcinogenicity of shale oils.' (Shale oils, IARC Volume 100F, p 204).

Shale oils are obtained from oil shale containing sedimentary rocks through a complicated industrial process. Shale oils are a very complex mixture of hydrocarbons and polar components. Shale oils have been causally linked to skin cancer, particularly in the scrotum, in humans. Epidemiological studies of risk for other sites are incomplete.

Shale oils are genotoxic in experimental systems. Shale oils were highly mutagenic in *S. typhimurium* in the absence of an exogenous metabolic activation system and demonstrated mutagenic activity in bacteria, fungi, and mammalian cells in culture.

Human studies showed conflicting results on genotoxicity of shale oil. A small group of workers showed increased chromosomal breakage levels but the workers were also involved with coke production. Elevated levels of DNA adducts have not been shown but the study groups have been small.

Soot, as found in Occupational Exposure of Chimney Sweeps (Volumes 3, 35 & Supplement 7)

'Extracts of soots contain carcinogenic polycyclic aromatic hydrocarbons and are genotoxic. Based on a small number of genotoxicity studies in exposed humans, there is moderate evidence of a genotoxic mode of action for the carcinogenic hazards associated with occupational exposures as a chimney sweep. The detection of anti-benzo[a]pyrene-7,8-diol-9,10-epoxide-DNA adducts in the peripheral blood lymphocytes of chimney sweeps suggests involvement of benzo[a]pyrene in the genotoxic effect of this exposure in humans.' (Soot, as found in occupational exposure of chimney sweeps, IARC Volume 100F, p 213)

Chimney sweeps are exposed to soot during their work. Soot is the by-product of combustion of various organic materials. The chemical composition is highly variable, depending on the material being burned and the conditions of combustion. Most soots contain a substantial amount of PAHs. The carcinogenicity of soots was first established by Percival Pott in 1775. They cause skin cancer (primarily of the scrotum) and lung cancer.

The PAH components of soot are believed to contribute towards carcinogenicity through the genotoxic mechanisms that have been discussed elsewhere. Experimental studies on soots have shown mutagenicity. Extracts of soot samples from domestic sources were mutagenic in *S. typhimurium*, both in the presence and absence of an exogenous metabolic system. Furthermore, extracts of particulate emissions from wood-combustion induced sister chromatid exchange in Chinese hamster ovary cells, transformation of Syrian hamster embryo cells, and mutation in *S. typhimurium*. Occupational exposures have been linked to modestly increased levels of DNA adducts and higher frequency of micronuclei.

Occupational Exposure during Aluminum Production (Volumes 34, 92 & Supplement 7)

'Air-emission samples from aluminum smelters were mutagenic in bacteria. There were mixed reports on the mutagenicity of urine from exposed workers. DNA-adduct studies of blood samples from aluminum-smelter workers also gave mixed results. Based on both experimental and human studies, there is weak-to-moderate evidence for a genotoxic mechanism underlying the effects of occupational exposures during aluminum production.' (Occupational exposure during Aluminum production, IARC Volume 100F, p 221)

Aluminum extraction from bauxite involves an electrolysis process using pre-baked anodes that are produced by moulding petroleum coke and coal-tar pitch in blocks. During electrolysis, the anode is consumed, exposing workers to PAHs from the coke and coal-tar pitch. PAH exposure has decreased steadily since the 1950's but still is the main carcinogenic agent to which workers are exposed. Aluminum production causes cancers of the bladder and lung.

Carcinogenic mechanisms related to coke and coal-tar pitch would apply to aluminum production when anode consumption occurs.

Air-emission samples from aluminum smelters were mutagenic in bacteria. Samples from air-filters in anode processing rooms were also mutagenic in bacteria. Human studies have found

mixed results for the mutagenicity of urine from exposed workers and the presence of DNA-adducts in workers.

Aflatoxins (Volumes 1, 10, 42, 56, 82 & Supplement 7)

'There is strong evidence that the carcinogenicity of aflatoxins operates by a genotoxic mechanism of action that involves metabolic activation to a genotoxic epoxide metabolite, formation of DNA adducts, and modification of the TP53 gene. In human hepatocellular carcinoma from areas where exposure to aflatoxins is high, up to 50% of tumours have been shown to harbour a specific point mutation in the TP53 tumour-suppressor gene.' (Aflatoxins, IARC Volume 100F, p 244)

Aflatoxins are produced by several species of the *Aspergillus* family of fungi. The fungus grows as a contaminant on foodstuffs, primarily maize, peanuts and cottonseed. Aflatoxins cause hepatocellular carcinoma. Concurrent infection with Hepatitis B increases the cancer risk.

Aflatoxins are genotoxic but require metabolic activation by members of the CYP450 family of enzymes. Initial metabolism creates the reactive *exo*-epoxide which then produces a DNA adduct. *'Aflatoxin B1 is the most common and potent of the aflatoxins. It is metabolized predominantly in the liver to an AFB1–8,9-exoepoxide, which forms a pro-mutagenic AFB1-N7-guanine DNA adduct that results in G→T transversion mutations. In human hepatocellular cancers in areas where aflatoxin exposure is high, up to 50% of tumours have been shown to harbour a specific AGG→AGT point mutation in codon 249 of the TP53 tumour-suppressor gene (codon 249Ser mutation)*'. (IARC Volume 100F, p 244)

Exposure to aflatoxin induces adducts to DNA and albumin, gene mutations and chromosomal alterations including micronuclei and sister chromatid exchange, and mitotic recombination. DNA and protein adducts of aflatoxin have been detected in many studies of human liver and body fluids. Aflatoxin induces mutations in *S. typhimurium* strains TA98 and TA100 but requires rat-liver S9 activation.

Benzene (Volumes 7, 29, 42 & Supplement 7)

'There is strong evidence that benzene metabolites, acting alone or in concert, produce multiple genotoxic effects at the level of the pluripotent haematopoietic stem cell resulting in chromosomal changes in humans consistent with those seen in haematopoietic cancer. .... [A] variety of genotoxic changes, including chromosomal abnormalities, have been found in the lymphocytes of workers exposed to benzene.' (Benzene, IARC Volume 100F, p 285)

Benzene is an intermediary in the manufacture of a wide range of organic chemicals. It occurs naturally in petroleum products, including gasoline and has been used as an additive in non-leaded gasoline to raise the octane rating. Most benzene exposure occurs through industrial contact; the general public is exposed mainly through atmospheric pollution from gasoline (due to heavy traffic exhaust levels or fumes from gasoline stations) and cigarette smoke. Some exposure can occur through contaminated food and water. Benzene causes acute myeloid leukaemia (AML) and acute non-lymphocytic leukaemia.

Benzene requires metabolism to active agents (in particular, benzoquinones) to display carcinogenic potential. Benzene induced leukaemia probably begins as a mutagenic event in stem or progenitor cells; subsequent genomic instability allows for sufficient mutations to be acquired in a relatively short time. Volume 100F lists eight genetic pathways to the development of AML (figure 4.2, p 280). Benzene has been linked to five of these pathways.

'Benzene may act by causing chromosomal damage (aneuploidy, deletions and translocations) through inhibition of topoisomerase II, disruption of microtubules and other mechanisms; by generating oxygen radicals that lead to point mutations, strand breaks and oxidative stress; by causing immune system dysfunction that leads to decreased immune-surveillance; by altering stem-cell pool sizes through haematotoxic effects; by inhibiting gap-junction intercellular communication; and by altering DNA methylation and perhaps specific microRNAs.' (IARC Volume 100F, p 283)

Exposure to benzene is associated with typical chromosomal changes seen in AML (e.g. 5q-/-5 and t(8,21)) in peripheral blood cells of heavily exposed workers. The hydroquinone metabolite produces similar changes in human cell culture, but hydroquinone is not classifiable as to its carcinogenicity to humans (IARC Group 3) based on inadequate evidence in humans and limited evidence in experimental animals. Benzene induces chromosomal aberrations in bone-marrow cells of mice, rats and Chinese hamsters and micronuclei and sister chromatid exchange in mice. It induces chromosomal aberrations and mutations in human cells *in vitro.* Benzene inhibits the DNA-related enzyme topoisomerase II, which is essential for the maintenance of proper chromosome structure and segregation. The ability of benzene and/or its metabolites to induce epigenetic changes is unclear at this time.

Updated Information

Recent studies have shown that benzene exposure may be associated with altered DNA methylation both in humans and animals [189,190,191]. Benzene metabolites such as hydroquinone and p-benzoquinone can generate phosphorylated histones [192].

Bis(chloromethyl) Ether and Chloromethyl Methyl Ether (Technical Grade) (Volumes 4, 42 & Supplement 7)

'There is moderate to strong evidence that bis(chloromethyl)ether and chloromethyl methyl ether, powerful alkylating agents, operate by a genotoxic mechanism. This mechanism is likely to be similar to that of other strong alkylating agents, involving modification of DNA and resultant mutations.' (Bis(chloromethyl) ether and chloromethyl methyl ether, IARC Volume 100F, p 305)

Bis(chloromethyl) ether (BCME) and chloromethyl methyl ether (CMME) are closely regulated chemicals with limited use except for the synthesis of other chemicals in enclosed systems. They cause lung cancer.

BCME and CMME belong to the group of chloroalkyl ethers. They are rapidly hydrolysed to form hydrochloric acid, methanol and formaldehyde. The understanding of the carcinogenic mechanism of BCME and CMME is limited. Few studies of genotoxicity and cytotoxicity have been performed. However, both compounds are known to be strong alkylating agents indicating that these compounds likely operate by a genotoxic mode of action. *In vitro*, CMME enhanced virus-induced transformation of Syrian hamster embryo cells and elicited unscheduled DNA synthesis. Formaldehyde (one of the primary metabolites of BCME) may contribute to the carcinogenic mechanism but BCME is much more potent at causing cancer, suggesting that formaldehyde is not the dominant agent.

1,3 Butadiene (Volume 39, 42, 54, 71, 97 & Supplement 7)

'There is strong evidence that the carcinogenicity of 1,3-butadiene in humans operates by a genotoxic mechanism that involves formation of reactive epoxides, interaction of these direct

acting mutagenic epoxides with DNA, and resultant mutagenicity. The metabolic pathways for 1,3-butadiene in experimental animals have also been demonstrated in humans.' (1,3 Butadiene, IARC Volume 100F, p 333)

'This mechanism of action is based on the observations that butadiene-induced mutagenicity requires metabolic activation, and that the DNA-reactive epoxides formed during butadiene biotransformation are direct-acting mutagens.' (IARC Volume 100F, p 332-3).

1,3-Butadiene is used in the manufacture of synthetic rubbers and polymers. Exposure is mainly occupationally linked although there is some low-level air-borne exposure of the general public. 1,3-Butadiene causes cancer of the haematolymphatic system.

1,3-Butadiene requires metabolic activation for carcinogenesis. Figure 4.1 in Volume 100F provides a detailed summary of the metabolism of 1,3-butadiene (IARC Volume 100F, p 328). Metabolism of 1,3-butadiene produces a number of epoxide intermediaries which likely contribute towards carcinogenesis. 1,3-butadiene and its metabolites produces DNA adducts in animals and humans with the N7 position of guanine being the major target site. The metabolites are mutagenic and genotoxic at multiple tissue sites in mice and rats, and in a variety of other test systems. An AT-TA transversion was consistently found across all biological systems. K-Ras, H-Ras, p53, p16/p15 and β-catenin mutations were detected in mice treated with 1,3-butadiene. Laboratory controlled modulation of key determinants of 1,3-butadiene metabolism has been shown to moderate genotoxicity.

Updated Information

Butadiene induces altered DNA methylation and histone modifications in animals [193,194].

2,3,7,8-tetrachlorodibenzo-para-dioxin (Volumes 15, 69 & Supplement 7)

'There is strong evidence to support a receptor mediated mechanism that operates in humans for carcinogenesis associated with 2,3,7,8-tetrachlorodibenzo-para-dioxin, where the primary mechanism is the promotion of tumour development through modification of cell replication and apoptosis, with a secondary mechanism related to increases of oxidative stress causing DNA damage. ....There is strong evidence to support a receptor-mediated mechanism for 2,3,4,7,8-pentachlorodibenzofuran- and 3,3′,4,4′,5-pentachlorobiphenyl-associated carcinogenesis....showing activity identical to 2,3,7,8-tetrachlorodibenzo-paradioxin (TCDD) for every step of the mechanism described for TCDD-associated carcinogenesis in humans.' (2,3,7,8-tetrachlorodibenzo-para-dioxin, 2,3,4,7,8-pentachlorodibenzofuran and 3,3′,4,4′,5-pentachlorobiphenyl, IARC Volume 100F, p 371)

2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD) has no commercial applications. TCDD is created as a contaminant in herbicide production. It is ubiquitous in the environment. TCDD exposure has been causally linked to an increase in overall cancer risk, rather than to risk at specific sites; the largest risk appears to relate to lung cancer, soft-tissue sarcomas and non-Hodgkin lymphoma.

TCDD is not directly genotoxic but carcinogenesis is mediated through interaction with the AhR receptor. TCDD produces sustained AhR activation due to a very long in-body half-life and sustained environmental exposure. Binding to AhR leads to the modification of the activity of a very wide range of Phase-I and –II genes. Through pathway cross-talk, activation also affects other receptor mediated pathways such as the oestrogen receptor and retinoic-acid receptor β.

This can lead to up-regulation of the enzymes and the enhanced production of reactive intermediaries from xenobiotic metabolism, which increases oxidative stress.

'TCDD has been shown to increase cellular proliferation both in vivo and in vitro in several tissues possibly through interactions with protein-kinase C signalling, inhibition of senescence or activation of growth-signalling factors' [6, p. 366].

2,3,4,7,8-pentachlorodibenzofuran (Volumes 15, 69 & Supplement 7)

The mechanistic summary from IARC is presented in the earlier section on 2,3,7,8-tetrachlorodibenzo-para-dioxin. (2,3,7,8-tetrachlorodibenzo-para-dioxin, 2,3,4,7,8-pentachlorodibenzofuran and 3,3′,4,4′,5-pentachlorobiphenyl, IARC Volume 100F, p 371)

2,3,4,7,8-pentachlorodibenzofuran (PCDF) has no commercial applications. PCDF occurs as a by-product of some combustion processes. It is ubiquitous in the environment. A causal link for human cancer has not been established for PCDF.

PCDF is chemically similar to TCDD, also binds to the AhR receptor and has similar metabolic effects. The carcinogenic mechanisms are discussed in a previous section (2,3,7,8-tetrachlorodibenzo-para-dioxin). The mechanistic evidence was used by the IARC panel to support its classification as a Group 1 carcinogen.

3,3′,4,4′,5-pentachlorobiphenyl (Volumes 15, 69 & Supplement 7)

The mechanistic summary from IARC is presented in the earlier section on 2,3,7,8-tetrachlorodibenzo-para-dioxin. (2,3,7,8-tetrachlorodibenzo-para-dioxin, 2,3,4,7,8-pentachlorodibenzofuran and 3,3′,4,4′,5-pentachlorobiphenyl, IARC Volume 100F, p 371)

3,3′,4,4′,5-pentachlorobiphenyl (PCB) and related PCBs were used extensively as dielectric insulation fluids in transformers. Their use was banned in 1977. PCBs are ubiquitous in the environment. A causal link for human cancer has not been established for PCB.

PCB is chemically similar to TCDD, also binds to the AhR receptor and has similar metabolic effects. The carcinogenic mechanisms are discussed in a previous section (2,3,7,8-tetrachlorodibenzo-para-dioxin). The mechanistic evidence was used by the IARC panel to support their classification as Group 1 carcinogens.

Ethylene oxide (Volume 11, 36, 42, 60, 97 & Supplement 7)

'There is strong evidence that the carcinogenicity of ethylene oxide, a direct-acting alkylating agent, operates by a genotoxic mechanism. A dose-related increase in the frequency of ethylene oxide-derived haemoglobin adducts has been observed in exposed humans and rodents, and a dose-related increase in the frequency of ethylene oxide-derived DNA adducts has been demonstrated in exposed rodents. Ethylene oxide consistently acts as a mutagen and clastogen at all phylogenetic levels, it induces heritable translocations in the germ cells of exposed rodents, and a dose-related increase in the frequency of sister chromatid exchange, chromosomal aberrations and micronucleus formation in the lymphocytes of exposed workers.' (Ethylene oxide, IARC Volume 100F, p395-6).

Ethylene oxide is primarily used as a raw material in the production of a wide-range of consumer products. It also has a role in the sterilization of medical devices and similar items. Most human exposure is occupational although some exposure occurs from air pollution and tobacco smoking. There has been no conclusive causal link between ethylene oxide and any

human cancer although there are suggestive links with lymphatic and haematopoietic cancers. Classification as a Group 1 carcinogen is largely based on evidence of carcinogenesis in animals and on the strong mechanistic evidence.

There is an extensive literature on the metabolism and carcinogenic mechanisms for ethylene oxide. It has been shown to be genotoxic and mutagenic in numerous assays and cell types. Increases in gene mutations and chromosomal alterations have been observed in multiple systems. *In-vitro* and *in-vivo* studies have shown that ethylene oxide can bind to cellular macromolecules, which results in a variety of DNA, RNA and protein adducts. Dose-response related increases in sister-chromatid exchanges and chromosomal aberrations have been observed in workers. *In-vivo* studies in mice and rats using mutation-induction with reporter genes such as Hprt or the LacI-transgene have shown a significantly increased mutation frequency.

Formaldehyde (Volumes 29, 62, 65, 66, 88 & Supplement 7)

'The current data strongly indicate that genotoxicity plays an important role in the carcinogenicity of formaldehyde in nasal tissues in humans, and that cellular replication in response to formaldehyde-induced cytotoxicity promotes the carcinogenic response. Three possible mechanisms, all focused around genotoxicity, are moderately supported as the underlying mechanism for induction of haematological malignancies in humans. Further research is needed to decide which of the mechanisms is the most important.' (Formaldehyde, IARC Volume 100F, p 430).

Formaldehyde is extensively used in industrial processes for the production of resins and other chemicals. It is also used in aqueous form as a disinfectant and preservative. It is also found in natural products (e.g. certain foods), cigarettes, automobile exhausts, etc. Formaldehyde causes cancer of the nasopharynx and leukaemia.

Formaldehyde has a genotoxic mechanism of action for the production of nasopharyngeal cancer. Micronucleus formation has been found in cells of the nasal and oral mucosa of formaldehyde-exposed humans. DNA–protein crosslinks have been observed in circulating white blood cells of exposed workers. Sister chromatid exchange, micronuclei formation and chromosomal aberrations have been observed in some studies of chronically exposed workers.

Studies in laboratory animals inhaling formaldehyde have shown genotoxic effects in the nasal tissues. DNA strand-breaks were induced by formaldehyde *in vivo*, in mouse liver and in rat lung cells. There is consistent *in vitro* evidence of formaldehyde-induced mutations, encompassing both clastogenic effects and direct DNA mutation. Formaldehyde showed mutagenic potential in several bacterial systems, both with and without S9 activation. Formaldehyde induced deletions, point mutations, insertions, and cell transformation in *in-vitro* assays with mammalian cells. Chromosomal aberrations, micronuclei and SCE were all increased *in vitro* in numerous rodent and human primary cells and cell lines treated with formaldehyde.

There is less understanding of the mechanism of carcinogenesis for leukemia, with concerns raised about whether inhaled formaldehyde can reach the bone marrow, which would be necessary for a direct carcinogenic mechanism to occur.

Updated Information

A recent study showed that formaldehyde induced global genomic hypomethylation in 16HBE cells 9 [195] and induced changes in micro RNA expression profiles in humans [196,197].

Sulfur Mustard (Volumes 9, 42 & Supplement 7)

'Data from a variety of sources all strongly support a genotoxic mechanism underlying the carcinogenic action of mustard gas/sulphur mustard, mainly based on the observation that this chemical is a bi-functional alkylating agent....The direct reaction of this substance with DNA likely initiates a cascade of genetic events that lead to cancer. There is evidence to support DNA-alkylation leading to cross-link formation, inhibition of DNA synthesis and repair, point mutation, and induction of chromosome-type and chromatid-type aberrations.... In addition, production of reactive oxygen species and cytotoxicity, other reported contributors to the mechanism of action, could act complementary to DNA alkylation. ' (Sulfur Mustard, IARC Volume 100F, p 446)

Sulfur mustard (also known as mustard gas) has been used as a chemical warfare agent in various military conflicts. Sulphur mustard is acutely toxic and can cause death within three days of exposure. At lower persistent exposure levels, it causes lung cancer.

Sulfur mustard (1,1'-thiobis (2-chloroethane) is an alkylating agent that produces DNA interstrand cross-linkages. *In vitro* studies have found that sulphur mustard induces DNA adducts in human cells and various animal models. Alkylation by sulphur mustard affects transcriptional processes and may lead to truncated transcripts by impairing RNA polymerase via an alkylated promoter. Cells in late G1 or S phase are at highest risk. Cells engaged in proliferation following tissue injury are particular targets.

Humans exposed to sulphur mustard from leaking munitions have demonstrated elevated rates of sister chromatid exchange. Animal studies have found chromosomal aberrations, micronuclei and mutations. Sulfur mustard can produce free-radical-mediated oxidative stress. It also inhibits anti-oxidant enzyme activity.

Vinyl Chloride (Volumes 7, 19, 42, 97 & Supplement 7)

'Numerous studies on the toxicokinetics, metabolism, genotoxicity, and molecular biology of vinyl chloride provide strong evidence that the carcinogenicity of this chemical involves a genotoxic mechanism of action, mediated by reactive metabolites....(K)ey events in the pathway of vinyl chloride-induced liver carcinogenesis....include metabolic activation to reactive metabolites, binding of the metabolites to DNA, pro-mutagenic action of these adducts leading to G→A and A→T transitions, and the effects of such mutations on the functioning of proto-oncogenes and tumour suppressor genes at the gene and protein levels.' (Vinyl chloride, IARC Volume 100F, p 472)

Vinyl chloride is mainly used as a precursor in the manufacture of polyvinyl chloride (a major plastic in worldwide use). Other uses were banned in 1974 in the USA. Non-occupational exposure is rare and at very low levels. Vinyl chloride causes angiosarcoma of the liver and hepatocellular carcinoma.

Vinyl chloride is rapidly absorbed by inhalation and is rapidly metabolized in the liver via a pathway subject to saturation. Chloroethylene oxide, a highly reactive compound, is a primary metabolite (for more details on metabolism, see IARC Volume 100F, p468).

The effect of vinyl chloride and its metabolites on cells is summarized succinctly in the IARC Volume 100F: *'Vinyl chloride is a genotoxic carcinogen in animals and humans. It is mutagenic, usually in the presence of metabolic activation, in various assays with bacteria, yeast or mammalian cells; it is also clastogenic in vivo and in vitro. Vinyl chloride induces unscheduled DNA synthesis, increases the frequency of sister chromatid exchange in rat and human cells, and*

increases the frequency of chromosomal aberrations and micronucleus formation in mice, rats, and hamsters in vivo....Chloroethylene oxide and chloroacetaldehyde can form etheno adducts with nucleic acid bases in vitro..' (IARC Volume 100F, p 470-1).

Polymorphic variations in metabolic genes (e.g. the CYP450 family) or DNA repair genes may alter carcinogenicity but do not affect the underlying mechanisms.

Isopropyl Alcohol Manufacture by the Strong-acid Process (Volumes 15 & Supplement 7)

'Little information on possible mechanisms of carcinogenicity of inorganic acid mists is available. The increased incidence of cancer of the paranasal sinuses in workers involved in the strong-acid process of isopropyl alcohol manufacture may be due to exposure to the strong acid mists and/or the presence of diisopropyl sulphate, an intermediate that shows sufficient evidence of carcinogenicity in experimental animals. Available data suggest that localized low pH from inhalation of inorganic acid mists could damage DNA and lead to neoplasia. There is no evidence that would support the occurrence of DNA damage by any other mechanism of carcinogenesis.' (Isopropyl alcohol manufacture by the strong-acid process, IARC Volume 100F, p483)

IARC has concluded that the manufacture of isopropyl alcohol by the strong-acid process is a Group 1 human carcinogen. However, isopropyl alcohol as a discrete entity was not classified as a human carcinogen. The primary route of exposure is through inhalation of an acid mist. The strong-acid process causes cancer of the nasal cavity.

There is limited direct evidence concerning a carcinogenic mechanism. It is hypothesized that local alterations in pH due to inhalation of the low-pH acid mist could lead to DNA damage through depurination of DNA and deamination of cytidine. Low pH may also alter the integrity of enzymes. However, direct laboratory support for this hypothesized mechanism is currently lacking. In addition, estimation of the impact of exposure to acid-mists on tissue pH levels is complex due to variation in droplet size, inhalation patterns, etc.

A small study of workers were found to have elevated levels of sister chromatid exchange (SCE), micronucleus formation and chromosomal aberrations in peripheral lymphocytes.

Updated Information

Isopropyl alcohol may induce immunosuppression in human cells *in vitro* [198,199] and interfere with inflammatory response in rats [200].

Mists from Strong Inorganic Acids (Volume 54)

The IARC mechanistic information related to Mists from Strong Inorganic Acids was presented in the previous section on Isopropyl alcohol manufacture by the strong-acid process. (Isopropyl alcohol manufacture by the strong-acid process [6, p. 483])

This exposure was discussed in the previous section (Isopropyl alcohol manufacture by the strong-acid process). Mists from strong inorganic acids cause lung cancer.

It is hypothesized that the same mechanism is likely in operation as for isopropyl alcohol manufacture by the strong-acid process. As noted in that section, there is a lack of a clear mechanism but it is hypothesized to be related to low-pH tissue exposure.

Updated Information

See section on isopropyl alcohol.

Occupational Exposures during Iron and Steel Founding (Volumes 34 & Supplement 7)

'There is moderate evidence that extracts of particles collected from a steel foundry act via a genotoxic mechanism, based on bacterial mutation studies. There is weak evidence for a genotoxic mechanism of action for exposures during iron and steel founding, based on DNA-adduct studies.' (Occupational exposures during iron and steel founding, IARC Volume 100F, p505)

'Founding' involves the production of steel or iron castings by re-melting ingots and shaping them using moulds. Workers are exposed to a range of substances including silica, carbon monoxide, airborne polycyclic aromatic hydrocarbons, nickel, chromium and formaldehyde. Foundry work causes lung cancer.

Cancer risk and carcinogenic mechanisms reflect those of the specific substances to which workers are exposed. In several studies, extracts of particulates from samples collected at a steel foundry or from air filters were mutagenic in *S. typhimurium* strain TA98 in the presence or absence of an exogenous metabolic activation system. In workers in a Finnish iron foundry, there was a significant positive correlation between the estimated exposure levels and aromatic DNA-adduct levels in peripheral white blood cells.

Updated Information

Studies in animals have shown that exposures to pollutants from iron and steel founding can induce mutations [201,202,203].

Occupational exposure as a painter (Volumes 47 & 98)

'The multiple genetic and cytogenetic effects observed among workers employed as painters or in the paint industry provide strong evidence in support of genotoxicity as one mechanism underlying the observed increase in cancer risk. However, due to the complexity and changing nature of the exposure mixtures and the potential interactions between exposures as a painter, other mechanisms are also likely.' (Occupational exposure as a painter, IARC Volume 100F, p 531)

Painting involves exposure to a diverse range of chemicals, including components of the paint (Pigments, dyes, binders, solvents and additives) and from preparation of the surface being painted. IARC estimates that painting involves over a thousand different substances. Many of these agents are potentially carcinogenic. Occupational exposure as a painter has been causally linked to mesothelioma, and cancers of the urinary bladder and lung.

Carcinogenic mechanisms reflect those of the specific chemical exposures. Many of these have been discussed in other sections of this chapter.

Painters have been found to have elevated levels of chromosomal aberrations, sister chromatid exchanges and micronuclei in buccal cells and culture lymphocytes. A positive association was noted with dose of exposure. Increased level of DNA strand breaks and DNA adducts have also been reported in painters.

Occupational exposures in the rubber-manufacturing industry (Volume 28, 42 & Supplement 7)

'The multiple genetic and cytogenetic effects observed among workers employed in the rubber manufacturing industry provide strong evidence to support genotoxicity as one mechanism for the observed increase in cancer risks. However, due to the complexity and changing nature of the exposure mixture and the potential interactions between exposures in the rubber-manufacturing

industry, other mechanisms are also likely to play a role.' (Occupational exposures in the rubber-manufacturing industry, IARC Volume 100F, p 559)

Workers in the rubber manufacturing industry are exposed to a wide variety of chemicals, the composition of which varies across industrial sites and sectors but originate from dusts and fumes created during the rubber-making and vulcanization processes. Potential exposures include N-nitrosamines, polycyclic aromatic hydrocarbons, solvents, and phthalates. Inhalation is the main route of exposure, although workers may have dermal exposure as well (e.g. to cyclohexane-soluble compounds). Occupational exposure in the rubber-manufacturing industry has been causally linked to leukaemia, lymphoma, and cancers of the urinary bladder, lung, and stomach.

Carcinogenic mechanisms reflect those of the specific chemical exposures. Several studies have found evidence of chromosomal aberrations, sister-chromatid exchange, micronucleus formation, premature chromosome condensation, DNA breakage, DNA-adduct formation, mutagenicity in urine, and mutation in the HPRT gene. For each of these endpoints, in most studies a positive response has been observed in exposed workers compared with non-exposed controls.

Post-Volume 100 Group 1 Human Carcinogens

Diesel Engine Exhaust (Volume 105)

'...there is strong mechanistic evidence that diesel engine exhaust, as well as many of its components, can induce lung cancer in humans through genotoxic mechanisms that include DNA damage, gene and chromosomal mutation, changes in relevant gene expression, the production of reactive oxygen species and inflammatory responses. In addition, the co-carcinogenic, cell-proliferative and/or tumour-promoting effects of other known and suspected human carcinogens present in diesel engine exhaust probably contribute to its carcinogenicity in the human lung. (IARC Volume 105, p 464).'

The diesel engine was invented in 1898 and has undergone extensive development leading to higher efficiency controls and emission control. Diesel engines power a wide range of automotive vehicles (including passenger cars, buses, ships and commercial vehicles) and also provide power for electricity generation sites. Occupational exposures occur for workers using these vehicles and machines. The general public is exposed to exhaust fumes from vehicles. Inhalation is the main route of exposure. Diesel engine exhaust is an established cause of cancer of the lung in humans and is linked to carcinoma of the bladder.

Diesel exhaust is a complex chemical mixture, containing gaseous and particulate components. The carcinogenic potential of diesel engine exhaust arises from these component chemicals, many of which have individually been classified as Group 1 or Group 2 human carcinogens by IARC. Components include: nitrogen oxides, sulphur, ozone, acrolein, benzene, formaldehyde, naphthalene, PAH's, lead, arsenic, and chromium. There is evidence that the carcinogenic effect is mainly related to the particulate components.

Carcinogenic mechanisms for diesel engine exhaust is complex, with component chemicals displaying strong evidence of a range of genotoxic effects including: sister chromatid exchange, mutation in bacterial and animal models, chromosomal aberrations, DNA adducts, DNA strand breaks and unscheduled DNA synthesis. PAH (including benzo[*a*]pyrene) and nitro-PAH are components with strong carcinogenic potential. They require activation by phase 1 metabolic enzymes but lead to DNA adduct formation. Mechanistic aspects for benzo[*a*]pyrene were discussed in an earlier section of this report.

Exposure to whole diesel engine exhaust produced increased levels of sister chromatid exchange and bulky DNA adducts. In lung tissue, exposure to particulates from diesel engine exhaust induces a strong immunological response, characterized by production of Reactive Oxygen Species and reactive oxygen DNA damage. Levels of pro-inflammatory mediators are increased with evidence for enhanced gene expression. Humans exposed to diesel engine exhaust develop airway inflammation and also excrete high levels of 1-hydroxypyrene, a marker of PAH exposure.

Trichloroethylene (Volume 63 & 106)

'In experimental animals and humans, oxidative metabolism of trichloroethylene is catalyzed by cytochrome P450 enzymes and GSH conjugation of trichloroethylene is catalyzed by GST enzymes. The formation of reactive metabolites of trichloroethylene in the kidney from processing of GSH-conjugation metabolites in situ has been observed in experimental animals and in human kidney

cells. The reactive GSH-conjugation metabolites of trichloroethylene are genotoxic on the basis of consistent results in several available test systems.' (IARC Volume 106, p 189).'

Trichloroethylene has industrial applications in the cleaning and degreasing of metal parts and as an intermediate in the production of other chemicals. It has minor uses as a spot remover in dry-cleaning and as a component of other consumer products. Its use has declined dramatically since 1970 due to environmental and health concerns and government regulations. Exposure is mainly through inhalation and is largely confined to occupational sites. Trichloroethylene causes cancer of the kidney and has been strongly linked to increased risk of non-Hodgkin lymphoma and liver cancer.

Trichloroethylene is rapidly absorbed following inhalation and is distributed throughout the body, mainly in tissues with a high lipid content. All active effects are due to metabolites of trichloroethylene. Of the two main metabolic pathways (CYP and GSH mediated), the GSH-conjugated metabolites are highly reactive while CYP metabolites are chemically stable. The initial GSH-conjugation occurs in the liver and produces *S*-(1,2-dichlorovinyl) glutathione (DCVG) which is subsequently metabolized in the kidney.

The carcinogenic mechanism for trichloroethylene exposure is unclear. Trichloroethylene is not genotoxic and is not a direct-acting mutagen. GSH-mediated metabolites (e.g. DCVG, dichloroacetic acid and chloral hydrate) are genotoxic, particularly in the kidney. Trichloroethylene and its metabolites also alter immune system function.

Limited studies in humans suggest that trichloroethylene exposed subjects may develop chromosomal aberrations and increased sister chromatid exchange although these effects may be due to GSH-metabolites or to co-exposure to stabilizing agents which are known mutagens. In bacterial, yeast and mammalian systems, trichloroethylene induces mutations but only following metabolic activation. *In vitro* and *in vivo* studies have found some evidence that exposure to trichloroethylene leads to chromosomal aberrations, micronuclei formation and sister chromatid exchange. Trichloroethylene is nephrotoxic in animals and human but experimental evidence linking this effect to the development of renal cell carcinoma is lacking. Animal and human evidence suggests that trichloroethylene has immunotoxic properties, increasing the risk of autoimmune disease; the role of this effect in carcinogenesis is unclear.

Working Group Carcinogenic Mechanism Summaries

As noted in an earlier section, each of the Working Groups which created the Volume 100 monographs for IARC were asked to provide a summary of the carcinogenic mechanisms for the agent in their report. These are summarized in Table 1 through 5. No mechanistic summaries were provided from volume 100D (radiation). Each table lists established carcinogenic mechanisms and, for some agents, lists other mechanisms that are likely involved but are not yet established. The depth of information varies across volumes: the Volume 100F mechanisms essentially just lists 'genotoxicity' and provide no details or indication of other mechanistic pathways which may be involved. However, for Volume 100C, multiple mechanisms are listed for most agents.

The information contained in the narrative summaries matches closely to the summary mechanisms provided by the Working Groups. However, the narrative summaries present some additional mechanisms than are listed by the Working Group, especially for agents listed in Volume 100F.

DISCUSSION

Important carcinogenic mechanisms can be identified for most of the Group 1 Human Carcinogens as identified by IARC. In some cases, the carcinogenic mechanism in humans is not clear (e.g. mineral and shale oils, Trichloroethylene). For some agents (e.g. 2,3,4,7,8-pentachlorodibenzofuran), the mechanistic evidence was so strong, that it formed a large part of the evidence base supporting the classification of the agent as a Group 1 human carcinogen.

Genotoxic mechanisms are the most common carcinogenic process identified through this narrative review. Of the 109 Group 1 human carcinogens considered in this review, 83 (76%) list genotoxicity as an element of their carcinogenic mechanism. A range of processes can lead to genotoxic events, including DNA adducts, DNA strand damage, and chemical changes to base pairs. All of these mechanisms are active in producing genotoxic effects.

There are Group 1 human carcinogens that do not have any genotoxic action but are still carcinogenic (e.g. most of the infectious agents listed in Volume 100B). Other common carcinogenic mechanisms include: oxidative stress, immunosuppression, chronic inflammation, stimulation of cellular receptors, epigenetic effects, and modifications of histones and other structural cellular elements. Other chapters in this monograph present schemas for characterizing the mechanisms in general categories. Initially, 24 toxicological end points were developed to classify carcinogenesis. However, this was later refined into 10 inclusive categories which capture the core carcinogenic mechanisms or processes.

Many Group 1 human carcinogens with established carcinogenetic mechanisms have multiple modes of action. While a genotoxic mechanism is very common, many agents have additional modes of action. For example, beryllium is genotoxic but also induces oxidative stress, increases cellular proliferation, alters cell-signalling pathways and modifies DNA repair. Diethylstilbesterol has genotoxic, receptor mediated and epigenetic effects that contribute to carcinogenesis. It is not always clear which mechanism is the predominant carcinogenic mechanism for an agent. Multiple modes of action may be required for carcinogenesis of some agents.

In preparing the narrative summaries, the focus was on the classes of carcinogenic mechanisms associated with an agent, rather than on specific genetic mutations created by these mechanisms and which participate in the cellular progression to cancer. For example, the IARC monographs and the database prepared for our project commonly list mutations to specific oncogenes (e.g. *p53*) as a mechanism. While mutations to these genes are directly linked to modifications in cellular function that lead to cancer, the action of the agent is to induce mutations, which will be randomly scattered through-out the genome. In the narrative summaries, we have emphasized the general class of action of the agent (production of mutations). This produces some apparent discrepancies between the details of the database and the narrative summaries. It would be useful to extend the exploration of mutations to identify the genetic drivers involved [204].

Many Group 1 human carcinogens share a commonality of carcinogenic mechanisms. Many of the chemical Group 1 carcinogens share a core chemical structure or function (e.g. they are alkylating agents). These agents have similar carcinogenic mechanisms.

Caution must be used in interpreting quantitative examination of carcinogenic mechanisms based on individual group 1 agents. The IARC Working Groups have sometimes classified a number of very similar agents as distinct Group 1 human carcinogens. This is most clear in Volume 100D (radiation) where distinct isotopes of radon, radium, etc. are classified as separate Group 1 carcinogens even though they are essentially the same agent, inducing carcinogenesis through a common mechanism related to radioactive decay. In contrast, other Working Groups have grouped similar agents into a single over-arching Group 1 agent. This was done for different isoforms of PCBs (IARC Monograph Volume 107) and for different sub-types of HPV. This could introduce a bias into a quantitative summary since some common mechanisms might be counted multiple times while others are only counter once. In subsequent chapters, we have grouped similar agents into a single group to avoid this issue.

A number of the Group 1 human carcinogens represent composite exposures or types of human behaviour. Tobacco smoking does not expose subjects to a single carcinogenic agent. Rather, there are over 100 known or suspected carcinogens contained within tobacco smoke. Many of the occupational exposures (e.g. painters) discussed in Volume 100F involve people being exposed to multiple potential or known carcinogens. In these cases, the carcinogenic risk and mechanisms would be expected to reflect those of the component exposures. Interactions among multiple exposures are possible and could lead to carcinogenic mechanisms not associated with the individual agents; these potential effects have not been widely studied.

The composite nature of many Group 1 human carcinogens presents challenges in summarizing the associated carcinogenic mechanisms. It is not feasible to report all of the potential mechanisms related to each known carcinogenic agent contained in the composite exposure. The narrative summaries attempted to capture the core mechanisms without providing details for each agent. In some cases, reference was made to other narrative summaries for agents (e.g. 'Betel Quid with Added Tobacco' was cross-referenced to the 'Tobacco Smoking' section).

Most of the scientific literature included in the IARC 100 volumes was published pre-2009, with many of the reports dating from 1995 or earlier. As a result, the mechanistic summaries reported in the IARC volumes would be expected to have an emphasis on mechanisms that received strong scientific study during that time period. Those summaries would also tend to contain less information about mechanisms that were identified more recently and may have not yet been widely studied for many agents (e.g. epigenetics, miRNAs). It is apparent from

reviewing both the narrative summaries and the Working Group Tables that genotoxic mechanisms feature prominently. The interpretation of this pattern needs to consider the potential for selection effects such as induced by the absence of research on non-genotoxic mechanisms for some agents. It would also be interesting to examine the rational for the process to examine the carcinogenicity of complex exposures and behaviours, as distinct from that of individual agents. While such composite agents have strong public health and policy implications, they contribute less to an understanding of carcinogenic mechanisms.

Table 1: Volume 100A Working Group Carcinogenic Mechanism Summaries

Agent	Established mechanistic events	Other likely mechanistic events
Busulfan	Genotoxicity, alkylating agent	
Chlorambucil	Genotoxicity, alkylating agent	Immunosuppression
Methyl-CCNU	Genotoxicity, alkylating agent	
Cyclophosphamide	Genotoxicity, bladder inflammation	Immunosuppression
Etoposide + cisplatin & bleomycin	Genotoxicity, translocations in genes	
Melphalan	Genotoxicity, alkylating agent	
MOPP	Genotoxicity	
Tamoxifen	Receptor-mediated, genotoxicity	
Thiotepa	Genotoxicity	
Treosulfan	Genotoxicity	
Diethylstilbestrol	Genotoxicity, ER-mediated events, including mitogenesis	Epigenetic programming (perinatal exposure)
Estrogen-only menopausal therapy	Receptor mediated, tissue specific, agent specific cell proliferation	Genotoxicity
E-P menopausal therapy, Combined	Receptor mediated, tissue specific, agent specific cell proliferation	Estrogen genotoxicity; stromal paracrine mediated effects
E-P contraceptives, Oral combined	Receptor mediated, tissue specific, agent specific cell proliferation	Estrogen genotoxicity; stromal paracrine mediated effects
Azathioprine	Immunosuppression, DNA damage	
Chlornaphazine	Metabolism to 2-naphthylamine derivatives,	

Agent	Established mechanistic events	Other likely mechanistic events
	alkylation(?)	
Cyclosporine	Immunosuppression	DNA damage (oxidative stress), DNA repair
Plants containing aristolochic acid	DNA adducts in humans A:T→T:A transversions in human tumours in p53	
Aristolochic acid	DNA adducts formed in animals are the same as those found in humans exposed to plants - A:T→T:A transversions in p53 - ras activation	
Methoxsalen+UVA	Genotoxicity following photo-activation	
Phenacetin	DNA damage DNA strand breaks in human cells, Chromosome aberrations, Proliferation in urothelia, bladder, renal pelvis	

Table 2: Volume 100B Working Group Carcinogenic Mechanism Summaries

Agent	Established mechanistic events	Other likely mechanistic events
EBV	Cell proliferation, Inhibition of apoptosis, Genomic instability, Cell migration	
HBV	Inflammation, Liver cirrhosis, Chronic hepatitis	Viral integration
HCV	Inflammation, Liver cirrhosis, Liver fibrosis	
KSHV	Cell proliferation, Inhibition of apoptosis, Genomic instability, Cell migration	
HIV-1	Immunosuppression (indirect action)	B-cell hyperactivation
HPV-16, 18	Immortalization, Genomic instability, Inhibition of DNA damage response, Anti-apoptotic	Inactivation/degradation of p53, Inhibition of Rb, others, Telomerase activation, PDZ binding,
HPV-33	Immortalization	Genomic instability, Inhibition of DNA damage response, Anti-apoptotic activity, Inactivation/degradation of p53, Inhibition of Rb, others, Telomerase activation, PDZ binding
HPV-31, 35, 39, 45, 51, 52, 56, 58, 59;	Immortalization (except for HPV-59)	Degradation of p53 (except for HPV-59)
HTLV-1	Immortalization and transformation of T-cells	
Opisthorchis viverrini	Inflammation, Oxidative stress	
Clonorchis sinensis		Inflammation,

Agent	Established mechanistic events	Other likely mechanistic events
(was Group 2A)		Oxidative stress
Schistosoma haematobium	Inflammation, Oxidative stress	Genotoxicity
Helicobacter pylori	Inflammation, Oxidative stress, Altered cellular turnover, Changes in gene expression, Methylation, Mutation	CagA translocation

Table 3: Volume 100C Working Group Carcinogenic Mechanism Summaries

Agent	Established mechanistic events	Other likely mechanistic events
Arsenic and inorganic arsenic compounds	Prevents cell-cycle blockage after genotoxicity, Cell proliferation	Resistance to apoptosis, Inflammation
Beryllium and beryllium compounds	Chromosome aberrations, Aneuploidy, Oxidative stress, DNA damage, Activation of proto-oncogenes, Apoptosis	Cell killing and compensatory cell proliferation
Cadmium and cadmium compounds	DNA repair inhibition, Genomic instability	
Chromium (VI) compounds	DNA damage, Mutations, Genomic instability, Aneuploidy	
Nickel compounds	DNA damage, Chromosome aberrations, Micronuclei, DNA repair disturbance, DNA methylation, Histone modification	Inflammation
Asbestos, all forms of (actinolite, amosite, anthophyllite, chrysotile, crocidolite, tremolite)	Genotoxicity, Aneuploidy and polyploidy, Inflammation, Tissue injury, Epigenetic alteration, Activation of signaling pathways, Resistance to apoptosis, Cell proliferation	
Erionite	See Asbestos	
Leather dust		
Silica, crystalline, in the form of quartz or crystobalite	Impaired particle clearance leading to macrophage activation and release of chemokines and cytokines	Free radical generation, Genotoxicity
Wood dust		

Table 4: Volume 100E Working Group Carcinogenic Mechanism Summaries

Agent	Established mechanistic events	Other likely mechanistic events
Tobacco smoking	Genotoxicity DNA adducts, Mutation in RAS, TP53, Tumour suppressor gene inactivation, Genomic instability, Loss of normal growth control, Cellular proliferation Key chemicals: arylamines, PAH, nitrosamines incl. NNN and NNK, volatile organics, others (cadmium, nickel, 210Po);	
Secondhand tobacco smoke	Genotoxicity (as tobacco smoking) Key chemicals: arylamines, PAH, nitrosamines incl. NNN and NNK, volatile organics, others	
Smokeless tobacco	Genotoxicity DNA adducts in human oral tissues, Chromosomal aberrations, Micronuclei, SCEs, RAS and TP53 mutations Key chemicals: nitrosamines incl. NNN and NNK, others;	Oxidative stress and ROS (high pH), Inflammation, Tumour-promotion Co-carcinogenesis
NNN + NNK	Genotoxicity, DNA-adducts in smokers and smokeless tobacco users, Micronuclei, Chromosomal aberrations, DNA strand break	
Betel quid with tobacco	Genotoxicity Key chemicals: nitrosamines incl. NNN and NNK, MNPN, NGL; areca-specific alkaloids, others	

Agent	Established mechanistic events	Other likely mechanistic events
Betel quid without tobacco	Genotoxicity Micronuclei, Chromosomal break Oral submucous fibrosis, Oxidative stress; 8-OH-dG DNA strand break. Key chemicals: areca-nut derived nitrosamines, MNPN, NGL; areca-specific alkaloids, others,	
Areca nut	Genotoxicity Areca-nut derived nitrosamine, MNPN causing DNA single strand breaks and DNA crosslinks in human buccal epithelial cells Accumulation of collagen->oral submucous fibrosis, Progression to oral cancer Key chemicals: areca-specific alkaloids (arecoline).	MNPN forms promutagenic DNA adducts and 2-(cyanoethyl) guanines in treated rats.
Alcohol consumption	Genotoxicity Effects due to ethanol and acetaldehyde	Oxidative stress, ROS, DNA strand break, Cirrhosis, Increase of the level of estrogen, inhibition of sex steroid-catabolism by increase redox state
Ethanol in alcoholic beverages	Chromosomal aberrations, Aneuploidy, Micronuclei Oxidation to acetaldehyde	
Acetaldehyde associated with consumption of alcoholic beverages	Acetaldehyde-derived-DNA-adducts, ALDH polymorphisms Chromosomal aberration, Aneuploidy, Micronuclei	
Chinese-style salted fish		Genotoxicity,

Agent	Established mechanistic events	Other likely mechanistic events
		Formation of nitroso compounds; Activation of EBV
Indoor emissions from household combustion of coal	Genotoxicity DNA adducts, TP53 and KRAS mutations in lung tumours Key chemicals: PAH, aromatic amines, nitrogen-containing heterocyclic aromatics, benzene, formaldehyde, arsenic, sulfur, silica);	Over loading deposited-particulates with associated sustained inflammation, ROS, Interstitial pulmonary fibrosis Chronic inflammation.

Table 5: Volume 100F Working Group Carcinogenic Mechanism Summaries

Agent	Established mechanistic events	Other likely mechanistic events
4-Aminobiphenyl	Genotoxic metabolites (strong)	
Benzidine	Genotoxic metabolites (strong)	
Dyes metabolized to benzidine	Genotoxic metabolites (strong)	
MOCA	Genotoxic metabolites (strong)	
2-Naphthylamine	Genotoxic metabolites (strong)	
o-Toluidine	Genotoxic metabolites (strong)	
Auramine production	Genotoxic metabolites (strong)	
Magenta production	Genotoxic metabolites (strong)	
Benzo[a]pyrene		
Coal gasification	Genotoxic (strong)	
Coal-tar distillation	Genotoxic (strong)	
Coal-tar pitches	Genotoxic	
Coke production	Genotoxic (strong)	
Mineral oils		
Shale oils		
Soot (as found in chimney sweeps)	Genotoxic (moderate)	
Aluminium production	Genotoxic (weak-to-moderate)	
Aflatoxins	Genotoxic epoxide metabolite (strong)	
Benzene	Genotoxic (strong)	
BCME, CMME	Genotoxic mechanism (moderate to strong)	
1,3-Butadiene	Genotoxic mechanism (strong)	
2,3,7,8-TCDD 2,3,4,7,8-PeCDF PCB 126	Receptor-mediated mechanism	
Ethylene oxide	Genotoxic mechanism (strong)	

Formaldehyde	Genotoxic and cellular
Sulfur mustard	Genotoxic (strong)
Vinyl Chloride	Genotoxic (strong)
Isopropyl alcohol manufacture	
Acid mists containing sulfuric acid	
Iron and steel founding	Genotoxic (weak)
Painting	Genotoxic (strong)
Rubber industry	Genotoxic (strong)

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